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EXPERIMENTS ON BODY-COOLING AND BODY-OVERHEATING IN BUFFALOES, SHEEP AND GOATS IN INDIA

By

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INTRODUCTION

A previous article (Minett, 1947) dealt with body-cooling following wetting of the skin by artificial showers, by natural rain or by immersion in water. The present paper gives an account of essentially practical experiments in buffaloes, sheep and goats on body-cooling due to exposure to cool breeze or to artificial showers, and overheating of the body following sun exposure.

In order to save space, mean values only are given for the voluminous data collected.

METHODS

Rectal temperatures were taken as far as possible by the same pair of observers, and care was taken that the thermometer was introduced as deeply as possible and held for a sufficient time. Respiration counts for at least two minutes were taken with the ear at the nostril in buffaloes and at the flank in sheep and goats.

RESULTS

Body-Cooling

The objects of these experiments were to obtain a clearer conception of the cooling effect due to shearing, to note how long the effect would last and to demonstrate the influence of slight exercise on the body temperature of freshly-shorn sheep.

Effect of Clipping

Two experiments were performed at Mukteswar. In the first, in August, 1942, when the daily maximum temperatures ranged from 60 to 70°F, 6 sheep of about the same size and weight were selected and 3 of them taken at random were closely clipped. The animals were held apart from one another in an open shed and their temperature recorded daily at 9 a.m., noon and 3 p.m. It is perhaps significant that two of the clipped sheep died during the experiment, after 7 and 19 days, and had to be replaced; partly owing to this mortality and partly because few animals were being used, the full results are not given. The experiment, however, was in line with the next one in showing that the body temperature of clipped sheep rises to the level of unclipped ones after about 20 days. The experiment also showed that the body temperatures of clipped sheep are

* *Dr. F. C. Minett died on 26th December, 1953. The article is published posthumously.*

lower than those of the unclipped, especially at the 9 a.m. readings; moreover, the clipped sheep showed far greater daily fluctuations.

The second experiment was carried out in April and May, 1943 and the result is shown in Chart 1. On April 20th, 15 unclipped sheep of similar weight, size and, so far as possible, condition were kept as a group in a shed and their temperatures recorded daily at 9 a.m. and 3 p.m., until May 5th when 10 of them were clipped. On the next day, the experiment proper began. Every day, just before 9 a.m. the unclipped sheep (group 2) and five clipped sheep (group 1) were carried from the shed to a dutch-barn nearby, being covered on the journey if raining. The five other clipped sheep (group 3), were given walking exercise every day from 8 to 9 a.m. finishing at the dutch-barn. There the sheep were tethered 4 or 5 feet apart, so that the individuals of each group were well separated. Their temperatures were recorded at 9 a.m., noon and 3 p.m. until May 28th. The dry and wet-bulb temperatures in the shed were recorded at 9 a.m. and 3 p.m. daily. Although the barn was in a sheltered spot, it was open at the sides and no rain could fall on the animals. At 3 p.m. they were taken back to the shed for the night. A concentrate ration (oil-cake, gram, barley, maize, bran) was fed at 8-30 a.m. and 3-30 p.m. while tree leaves were given as roughage. Water was withheld until the day's observations were completed. From Chart 1 it will be seen that the body temperature of the clipped unexercised sheep (group 1) followed a lower course than that of the unclipped (group 2) which remained fairly level. The difference was especially marked with the 9 a.m. readings and during the first 9 days of the experiment proper while by the 18th day after clippnig the curves approximated. On the other hand, there was a clear advantage from a little morning exercise for the mean temperature of sheep (group 3) was even higher than that of the unclipped animals.

Effect of Breeze on Buffaloes and Goats

Buffaloes. Ten buffalo bullocks, 6 to 7 years old, were divided into two lots of five. The plan was to raise their temperatures by walking exercise in a sheltered place and then to expose one group to the breeze while the other was to be sheltered by a screen. On the following day, the same procedure was to be followed with the groups reversed. Three experiments were conducted in this manner at Izatnagar in February, 1944, at which time the sunlight was not strong. Groups composed of the same individuals were used, except that in the third experiment one buffalo was replaced by a fresh animal. There was fairly close correlation between the bodyweights and body measurements of the individuals forming the groups; the mean weight in each case being approximately 700 lb. The exercise was given from 10 to 11 a.m. and the animals were then placed in shelter until noon, when the one group was fully exposed to the breeze for two hours, the individuals being tied four feet apart,

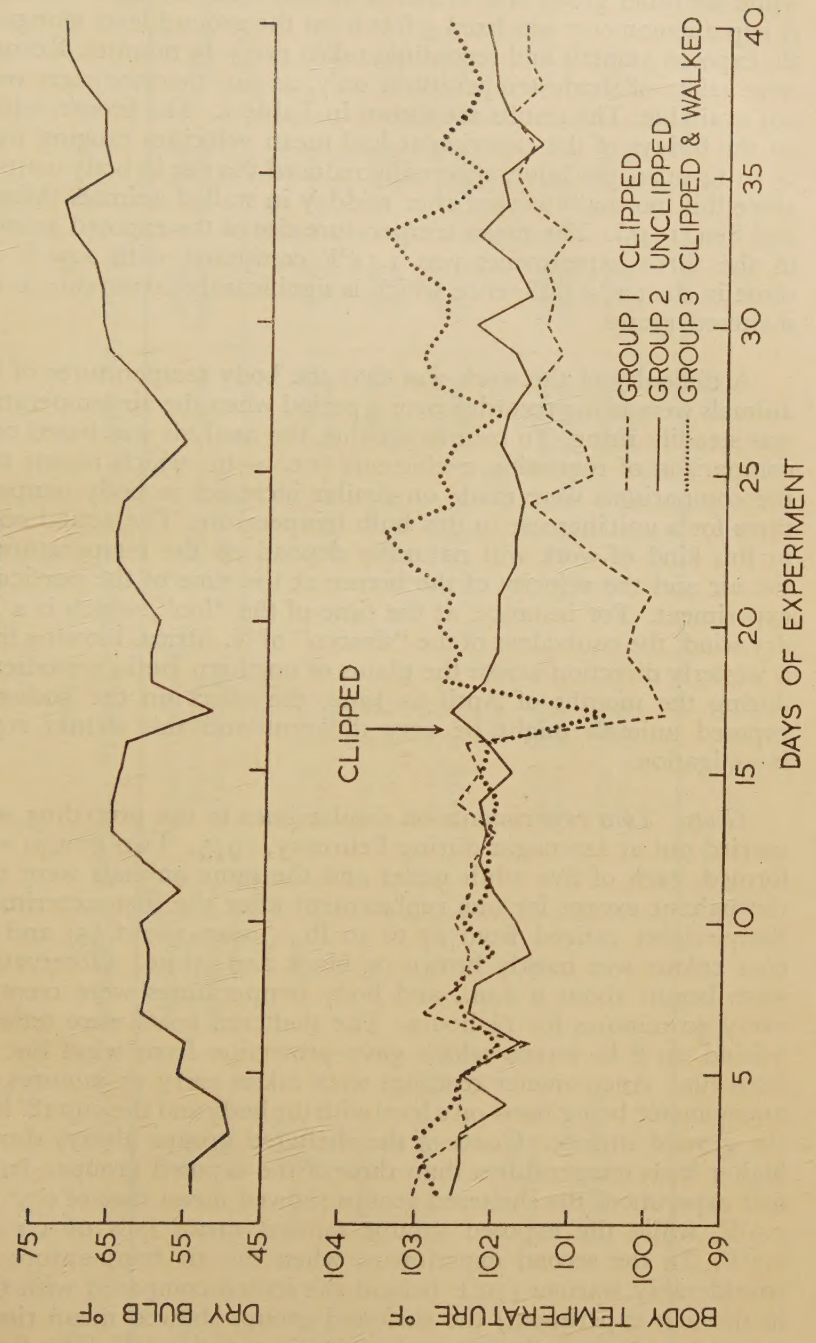


Chart 1. Means of 9 a.m. temperature readings of five sheep in each of three groups.

while the other group was sheltered behind a screen eight feet high. A cup anemometer was fixed 5 feet from the ground level alongside the exposed animals and recordings taken every 15 minutes. Records were taken of shade temperatures only, as sun thermometers were not available. The results are shown in Table 1. The breeze, which on the 6 days of the experiment had mean velocities ranging from 6½ to 14 miles per hour, effectually reduced the rise in body temperature that normally occurs after midday in stalled animals (Minett and Sen, 1945). The mean temperature rise of the exposed animals in the three experiments was 1.14°F compared with 2.94°F for those in shelter, a difference which is significantly favourable to the sheltered group.

A difficulty of the work was that the body temperatures of the animals were being recorded over a period when the air temperature was steadily rising. To counteract this, the analysis was based on a comparison of regression coefficients (r.c. = b), which means that the comparisons were made on similar increases in body temperatures for a unit increase in dry bulb temperature. The actual result in this kind of work will naturally depend on the temperature of the air and the velocity of the breeze at the time of the particular experiment. For instance, at the time of the "loo", which is a hot dry wind, the equivalent of the "sirocco" of N. Africa, blowing from a westerly direction across the plains of northern India periodically during the months of April to June, the effect on the bodies of exposed animals might be very different and this should repay investigation.

Goats. Two experiments on similar lines to the preceding were carried out at Izatnagar during February, 1945. Two groups were formed, each of five adult males and the same animals were used throughout except for one replacement after the first experiment. Bodyweights ranged from 27 to 40 lb., (mean about 35) and the coat colour was mostly brown or black and white. Observations were begun about 9 a.m., and body temperatures were recorded every 30 minutes for 1½ hours. The sheltered goats were tethered behind an 8 ft. screen which gave protection from wind but not from sun. Anemometer readings were taken every 15 minutes, the anemometer being fixed on a level with the body and the cup 18″ from the ground surface. Goats of the sheltered groups always showed higher body temperatures than those of the exposed groups. In the first experiment the sheltered groups showed mean rises of 0.5° and 1.0°F, while the exposed groups showed mean falls of 1.1 and 1.3°F. In the second experiment, when the air temperature was considerably warmer (70°F behind the screen compared with 61°F in the first experiment) the sheltered groups showed mean rises of 2.9 and 2.5°F and the exposed groups 0.3 and 0.1°F. The figures in Table 1 are overall averages, and in Experiment 2 relate to

TABLE 1

EFFECT OF BREEZE ON BODY TEMPERATURE OF BUFFALOES AND GOATS

Expt.	*Date*	*Duration of observations daily (hours)*	*Dry bulb (°F.) humidity Ranges approx. (first day, second day)*	*Wind velocity m.p.h.*	*Grouping*	*Initial body temp. °F.*	*Final body temp. °F.*	*Difference*
			Buffaloes					
	1944							
1	Feb. 2 and 3	2	67–74, 71–77	—	sheltered	98.27	101.46	3.19
			65–71, 69–75	9.8, 14	exposed	98.05	99.40	1.35
2	Feb. 4 and 5	2	67–65, 69–75	—	sheltered	97.80	100.70	2.90
			65, 67–73	6.5, 13	exposed	97.81	98.92	1.11
3	Feb. 27 and 29	2	70–77, 73–77	—	sheltered	98.15	100.88	2.73
			69–76, 72–76	13, 13	exposed	98.15	99.10	0.95
			Goats					
	1945							
1	Feb. 8 and 9	1½	54–62, 54–62	—	sheltered	102.40	103.10	0.70
			52–61, 52–62	8.4, 7.5	exposed	102.70	101.45	1.25
2	Feb. 23 and 24	3	58–74, 61–74	—	sheltered	101.25	104.80	3.55
			56–71, 57–70	8.5, 9.2	exposed	101.20	101.30	0.10

exposure for 3 hours, not 1½. The body temperature rise in the sheltered goats of the second experiment was sufficient to cause panting in three of them.

Considering the first experiment statistically there were highly significant differences between the body temperatures of the 10 goats both initially and after treatment, an effect which was almost entirely due to one feverish animal which was omitted from subsequent calculations. For the remaining 9 goats the pre-treatment means were not significantly different, being 102.64 for sheltered and 102.83 for exposed animals, each having S.E. ± 0.19. Similarly the body temperatures of the animals showed no significant differences after 1½ hours' treatment, but there was a highly significant difference, 1.47 ± 0.24, between the means (9) for sheltered, 103.17, and exposed, 101.70, each having S.E. ± 0.17.

The second experiment was first analysed for the same 9 goats which had been used in the previous experiment. As before no significant differences were found between the animals as regards body temperature either before or after treatment and no significant difference between the means (9) before being sheltered, 101.30, or exposed, 101.12, each having S.E. ± 0.17. After 1½ hours' treatment it was found as in the previous experiment that there was a highly significant difference, 2.68 ± 0.49, between the means of sheltered, 103.98, and exposed, 101.30, each having S.E. ± 0.35.

Since both experiments had 9 goats in common it was possible to compare the results obtained for these animals. It was found that the effect of exposure was much greater in Experiment 2 than in Experiment 1 but this could be explained by the difference in climatic conditions.

Considering in the second experiment the temperatures of all animals (the two groups of ten) when taken after three hours treatment, a preliminary analysis of variance showed there was no significant difference between animals but a highly significant difference between the behaviours of animals under the various treatments. The mean body temperature of those sheltered was now 104.84°F and of those exposed 101.31°F, each with S.E. ± 0.32. The difference was 3.53 ± 0.44.

Overheating of the Body

Buffaloes

Observations were made on a group of nine buffalo bullocks on May 14th, 15th, 16th and 20th, 1943 at Izatnagar. At this period shade temperatures were uniform, ranging from about 95°F at 10 a.m. to 109°F at 3 p.m. with strong sunlight. The object was to record every hour the body temperatures and respiration rates when the animals were (a) standing in the sun (i) without water (ii) with opportunity to drink every two hours from noon onwards (iii) with wetting of the body surface every two hours from noon and (b) standing in the shade without water. Temperature and

respiration records were also made 30 minutes after the wetting in group (a)(iii). The animals were fed at 6 a.m. and 6 to 7 p.m. and watered at 6-30 a.m. and 6 p.m. On other than experiment days they were also accustomed to take water at 12 noon. In the first

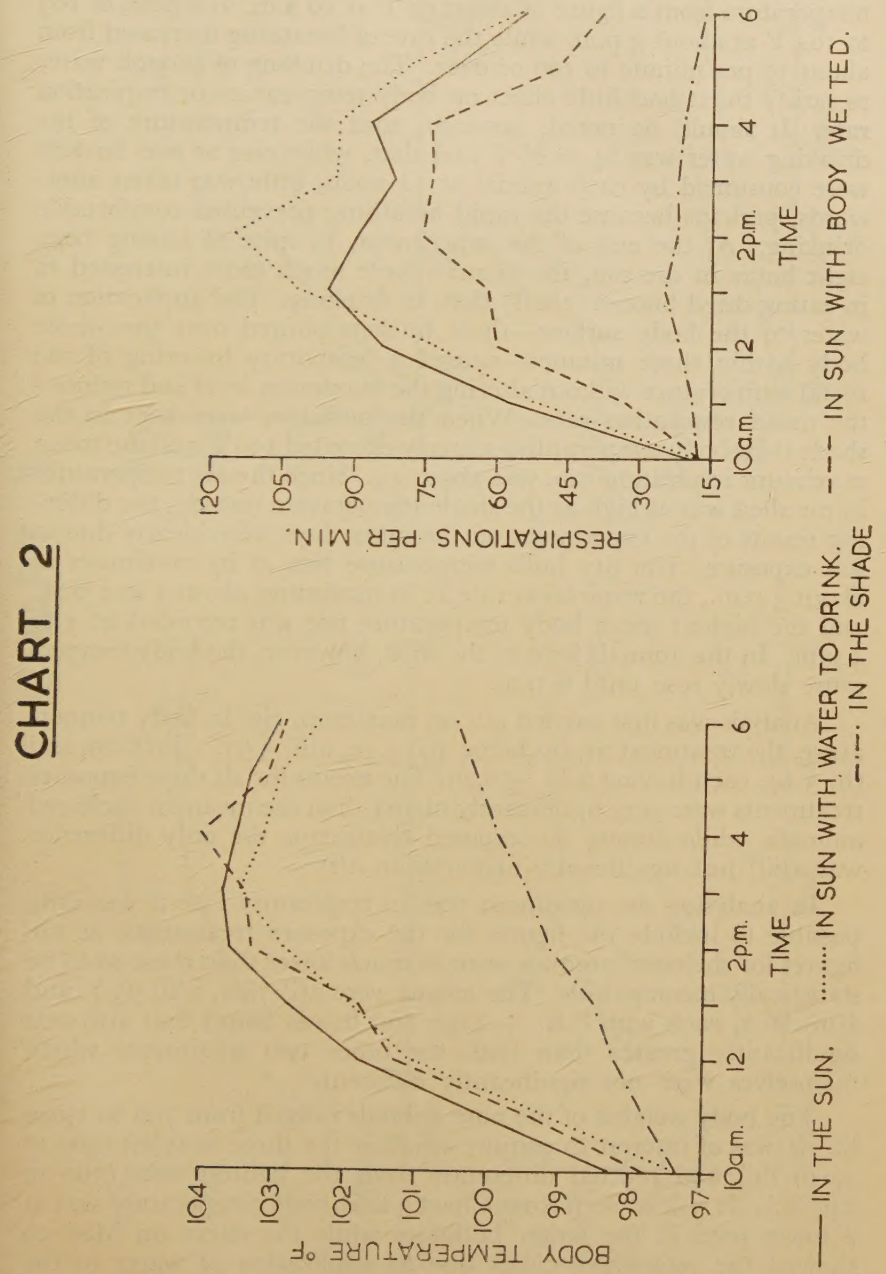


Chart 2. Means of body temperatures and respirations of nine buffaloes in each of four groups subjected to different treatments.

group the animals were tethered about four feet apart, in a small enclosure sheltered from the wind, from 10 a.m. to 6 p.m. : in the second they were kept in their shed. The results are shown in Chart 2. It will be seen that exposure to the sun raised the mean body temperature from a figure of about 98°F at 10 a.m. to a peak of 103 to 104°F at about 3 p.m. while the rate of breathing increased from about 19 per minute to 100 or over. The drinking of enough water to satisfy thirst had little effect on body temperature or respiration rate. It should be noted, however, that the temperature of the drinking water was 84 to 86°F and that, while one or two buckets were consumed by each animal at 12 noon, little was taken afterwards, perhaps because the rapid breathing prevented comfortable drinking. At the end of the experiment, in spite of having been eight hours in the sun, the animals were much more interested in in eating dried bhoosa (chaff) than in drinking. The application of water to the body surface—three buckets poured over the whole body within three minutes—caused a temporary lowering of the rectal temperature without altering the maximum level and reduced the mean respiration rate. When the buffaloes were kept in the shade their body temperatures scarcely exceeded 100°F and the mean maximum respiration rate was about 25. Since the air temperature in the shed was as high as the shade temperature outside, the differing results of the experiment on the other days were clearly due to sun exposure. The dry bulb temperature was at its maximum at about 3 p.m., the respiration rate at its maximum about 1 to 2 p.m. and the highest mean body temperature rise was recorded at 3 to 4 p.m. In the animals kept in the shed, however, the body temperature slowly rose until 6 p.m.

Analysis was first carried out on maximum rise in body temperature, the treatment means being a(i) 5.52, a(ii) 5.97, a(iii) 6.20, and (b) 3.14, each having S.E. $\pm$ 0.20. The means for all three exposure treatments were very significantly higher than the mean for sheltered animals, while among the exposed treatments the only difference was a(iii) just significantly higher than a(i).

In analysing the maximum rises in respiratory rate it was only possible to include the figures for the exposure treatments, as the figures for sheltered animals were so much lower than these as to be statistically incompatible. The means were a(i) 78.2, a(ii) 95.7, and a(iii) 66.2, each with S.E. $\pm$ 4.93, and it was found that a(ii) was significantly greater than both the other two treatments which themselves were not significantly different.

The body weights of the nine animals ranged from 700 to 1,040 lb. It was of interest to enquire whether the three heaviest (900 to 1,040 lb.) had reacted differently from the lightest ones (700 to 740 lb.). It will be seen from Chart 3 that body temperature was at a lower level in the larger buffaloes while the curve on May 20 showed the temporary check due to application of water to the

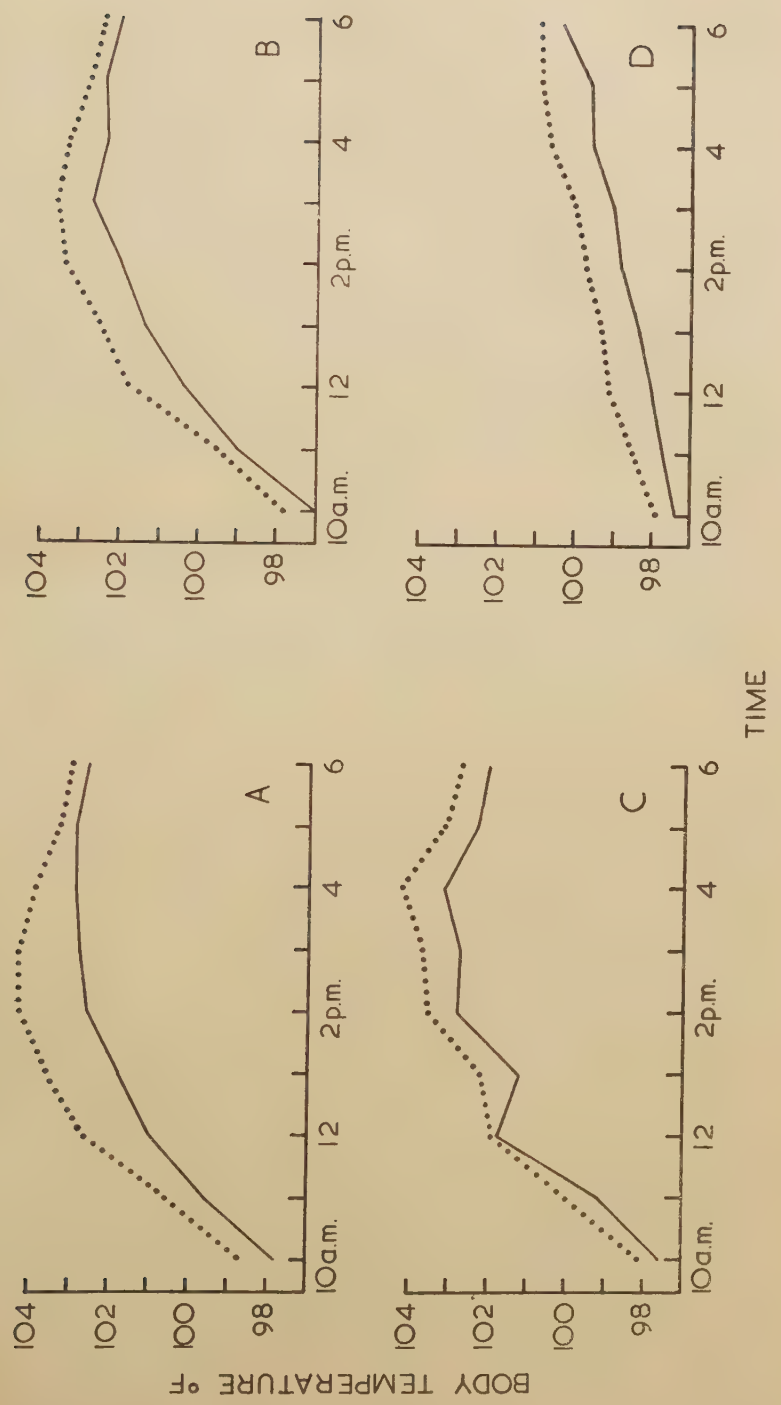


Chart 3. Means of body temperatures of three large buffaloes (solid line) and three small buffaloes (dotted line) subjected to different treatments : A in the sun, B in the sun with water to drink every two hours, C in the sun with water splashed on the body every two hours and D in the shade.

bodies. Blood taken on May 28th gave the following values:— haemoglobin 9.3, 9.5, 8.6; cell volume 35.2, 35.1, 31.4 for the three heaviest: haemoglobin 7.2, 7.1, 7.2; cell volume 26.7, 24.9, 25.3 for the three lightest. From this it appeared that the heavier buffaloes were in a better position to withstand the heat than the lighter ones, no doubt owing to their bigger chest capacity and the higher level of haemoglobin and blood cell volume. Unfortunately statistical comparison of the three heaviest and three lightest animals did not support these observations, no significantly different reaction to the treatments being detected for the two groups. With the respiratory rates the high variation among the heavy buffaloes was responsible for a high Standard Error and consequent loss of significance. Also, significant differences were noted among the buffalo means, even after allowing for differences in body weight, chiefly due to the presence in the experiment of one animal whose mean respiration rate was 20 less than any other.

Sheep and Goats

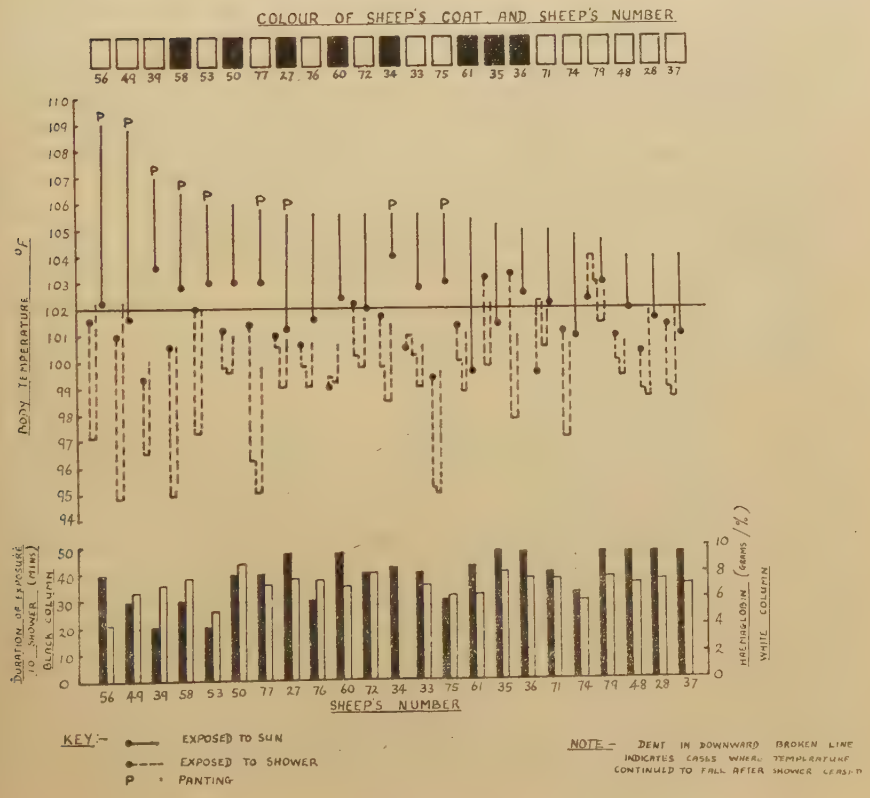
The object of the experiments on these species was to determine (a) whether the differences in individual animals in respect of body cooling were similarly manifested when the animals were overheated (b) the influence of coat colour and (c) the influence of body size on the overheated animal. During all these observations drinking water was withheld.

Sheep exposed first to artificial shower and later to hot sun. Twenty-four male sheep which had been clipped on the day before the experiment commenced were divided into three equal lots. At 8 a.m. on three consecutive days in March 1944 at Izatnagar, each group was exposed to an artificial shower limited to 48 minutes but some sheep were removed before that period had elapsed because their temperatures had fallen to 95 to 97°F. None however was exposed for less than 20 minutes. The temperature of the shower water was 66 to 69°F and the "rainfall" per hour was 3 to 3½ in. The air temperature during drying was 64 to 70°F. Temperatures were taken every half hour during a drying-off period of about four hours. Twenty days later the same animals were subjected to an overheating test. For this purpose they were tethered in the sun at a spot sheltered from wind, and not less than three feet apart, from 3 to 6 p.m. A note was made of the extent to which their rectal temperatures rose and of panting as a symptom of distress. The shade temperature was about 97°F. Any sheep whose temperatures reached 107 to 109°F were at once removed to the shade. Chart 4 shows the extent of the temperature swing for individual sheep from the minimum recorded during cooling, which was usually during the first half of the drying-off period, to the maximum recorded during overheating. Thus the animal on the left (49) showed a temperature swing of 14.2°F and the one on the right (79) 3.2°F,

the average being 7.6°F. It may further be noted that most of the sheep having the greater range of temperature showed obvious panting on exposure to the sun and that several of them were thin. It will also be seen that five of the animals on the left of the chart had haemoglobin values below 7.0 g. per cent. The mean haemoglobin value of those noted to pant was 6.45 g. per cent (4.2 to 7.6) compared with 7.45 g. (5.9 to 8.7) for the others. Actually, those animals subsequently noted to pant had only been given an average of 33 minutes exposure to the shower whereas the others had endured a mean exposure of 43 minutes. Similarly, it may be noted that the two animals showing the greatest temperature swing (49 and 56) could barely endure one hour of the sun bath. The indication from this experiment is that animals that are least able to stand exposure to cold are also least able to withstand excessive heat. This important conclusion is not quite borne out statistically. The correlation coefficient (r) between the maximum drop in shower and the maximum

CHART 4

SHEEP EXPOSED TO COOLING SHOWER AND LATER TO HOT SUN



rise in sun works out at +0.4078. (For significance at the five per cent. point it should be +0.413.) The actual mean maximum drop in body temperature after exposure to the shower was 2.49°F ±0.45 and the mean maximum rise on exposure to sun was 3.46°F ±0.30.

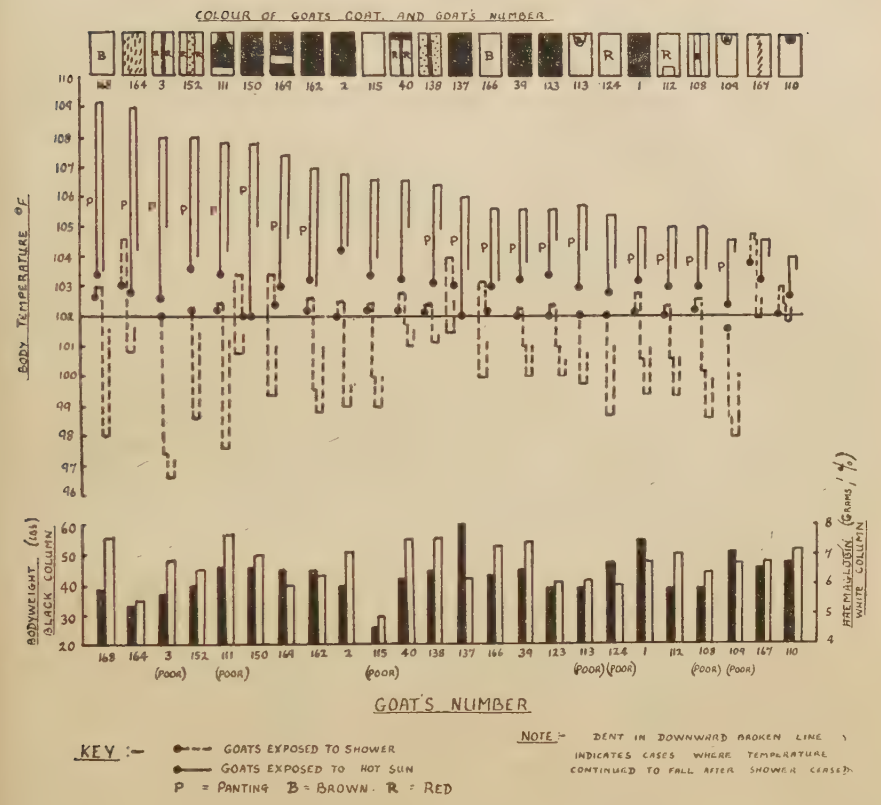
Other statistical calculations concerned coat colour and body weight. As regards the former, the mean maximum drop in body temperature after exposure to rain of white animals was 2.35°F ±0.57 and of black animals 2.76°F ±0.80. After exposure to sun, 15 white animals were compared with eight black ones. The mean maximum rise of the whites was 3.45°F ±0.40 and that of the blacks 3.48°F ± 0.45. These differences respectively are not significant.

Goats exposed first to artificial shower and later to hot sun. In April, 1945 at Izatnagar 24 animals (16 males and 8 females) were picked at random. They were in poor or moderate condition only and were exposed on April 3rd, 4th and 6th in batches of eight to a heavy artificial shower equivalent to about nine inches per hour, for seventy minutes at 8 a.m. each day. The temperature of the shower was 60 to 61°F and the air temperature during the drying period 78 to 84°F. Body temperatures were taken every 30 minutes during the rain and the three hours of the drying-off period. On April 12 the animals were tethered several feet apart in a screened enclosure from 1 to 7 p.m. The sun bath was somewhat severe as air temperatures ranged from 96 to 100.5 to 91.5°F and the mean anemometer speed during the exposure was 1.4 miles per hour. One animal (168) had to be placed in the shade after two hours exposure as its temperature had risen from 103.4 to 109.2°F. In this experiment also no clear relationship was seen between the extent of body temperature fall on cooling and the rise on sun exposure, any such relationship being presumably disturbed by the factor of coat colour (Chart 5). The relation of maximum drop to maximum rise in body temperature, as measured by r, was +0.274 which is not significant. The greatest range of body temperature in any one animal between cooling and heating was 11.4°F (goat 3) and the smallest 2.1°F (goat 110).

As regards the cooling effect of the shower, the following points were noted. (a) Within 15 minutes of the onset of the shower the body temperature rose in many cases (mean 0.6°F); in a few animals the rise was as much as 1 to 1.6°F. In many, the downward fall continued during drying. (b) Over the three groups taken together the mean drop in body temperature due to the shower was 1.94°F ± 0.33, this being significantly different from zero. The same degree of significance was shown between the mean initial body temperature and the mean maximum temperature recorded during the three hours drying period, viz. 1.26°F. (c) The goats in poor condition were among those showing the greatest falls of body temperature. Indeed in four cases as the fall was relatively precipitous it was not deemed expedient to give the full shower. (d) When the animals were

CHART 5

GOATS EXPOSED TO COOLING SHOWER AND LATER TO HOT SUN


COLOUR OF GOATS COAT AND GOAT'S NUMBER
B R B R R
164 3 152 111 150 169 162 2 115 40 138 137 166 39 123 113 124 1 112 108 109 167 110
BODY TEMPERATURE °F
110 109 108 107 106 105 104 103 102 101 100 99 98 97 96
P
BODYWEIGHT (lbs) BLACK COLUMN
60 50 40 30 20
HAEMOGLOBIN (GRAMS / %) WHITE COLUMN
8 7 6 5 4
164 3 152 111 150 169 162 2 115 40 138 137 166 39 123 113 124 1 112 108 109 167 110
(POOR) (POOR) (POOR) (POOR) (POOR) (POOR) (POOR)
GOAT'S NUMBER
KEY :- GOATS EXPOSED TO SHOWER
GOATS EXPOSED TO HOT SUN
P = PANTING B = BROWN R = RED
NOTE :- DENT IN DOWNWARD BROKEN LINE INDICATES CASES WHERE TEMPERATURE CONTINUED TO FALL AFTER SHOWER CEASED

ranged in order of body weight (mean of three consecutive daily weighings) it became apparent that the extent of body temperature fall tended to increase with decreasing weight. Thus, the ten heaviest goats (60 to 45 lb.; mean 49) showed a mean maximum fall of 2.41°F and the ten lightest (40 to 25 lb.; mean 37) a mean maximum fall of 3.26°F. The temperature differences however did not prove to be statistically significant ($r = -0.131$). The explanation may lie in the interaction of two factors viz, body weight and body condition, for three thin goats belonged to the heavy group and four to the light group. Coat colour did not enter into this particular problem. (e) No significant correlation existed between the maximum body temperature fall and the haemoglobin level of the animals

As regards the heating effect of the sun it was noted that the mean initial temperature of the 24 animals was 103.03°F and the mean maximum temperature reached 106.36°F, a rise of 3.325°F $\pm$ 0.30, significantly different from zero. Coat colour was of great

importance although in this experiment no purposeful selection for colour was made. Thus, on comparing nine mostly black with five mostly white goats a highly significant difference (at one per cent point) existed in respect of the mean maximum temperature reached on exposure to sun. This result did not show up clearly in the mean maximum rise owing to the fact that the black animals had a higher initial temperature than the white ones. The figures in question are as follows:—

Colour	*Initial Temp.*	*Max. Temp.*	*Rise*
Black	103.07 ± 0.23	106.56 ± 0.35	3.49 ± 0.44
White	102.86 ± 0.14	104.78 ± 0.28	1.92 ± 0.36

To test the influence of body weight upon initial body temperature and rise of temperature on exposure to sun, twelve light animals (mean 38.1 lb.; range 26 to 43) and twelve heavy animals (mean 48.4 lb.; range 45 to 60) were compared. The results showed a nearly significant (five per cent) negative correlation between body weight and initial body temperature (light 103.2°F and heavy 102.8°F). There was, however, no significant difference in the mean maximum rise of the two groups in the sun (light 3.54°F and heavy 3.11°F) or the mean maximum temperature reached (light 106.8°F) heavy 106.0°F).

Goats exposed first to warm sun and then to an artificial shower. In this experiment, carried out at Mukteswar in May, 1945 ten black and ten white goats were used. Apart from colour the groups were practically identical as the following values show :—

	Black	*White*
Bodyweight (lb.)	32.0	32.0
Height (in.)	19.8	19.6
Heart girth (in.)	21.8	21.3
Body length (in.)	20.4	20.3

The black females were heavier than the black males (34 : 29.8) and had larger heart girths (22.8 : 20.8). In these two respects the differences between white males and white females were negligible. The animals were tied in the sun in a fairly sheltered place though there was some wind after 1 p.m. Exposure was from 10.30 a.m. to 3.30 p.m. and the air temperature level, ranging from 71 to 75 to 72°F, was high enough to make many of the animals pant. Four days later the animals were tied in the shed and were subjected to water showers (at 64°F), on this occasion from a watering can, every few minutes from 9-50 to 10-50 a.m. Body temperatures were taken during the shower and during a four-hour drying period (air temperature, 69 to 71°F).

As regards the heating effect of the sun the following mean values were obtained :—

Colour	*Sex*	*Initial temp.*	*Max. temp.*	*Rise*
Black	F	102.08	105.66	3.58
	M	102.80	105.10	2.30
White	F	102.18	104.00	1.82
	M	102.76	104.68	1.92
Both colours and sexes		102.46 ±0.12	104.86 ±0.14	2.405 ±0.18

In respect of the initial body temperature there was no significant difference between black and white if sex difference is ignored. The male goats had significantly higher temperatures than the females (five per cent). Considering the maximum body temperature level reached it was found that the black goats were significantly higher than the white goats (one per cent). Difference between means of ten black goats and of ten white goats was 105.38—104-34 = 1.04 ± 0.28. Taking black and white goats together there was no significant difference between the sexes. Difference between means of ten males and of ten females was 104.89—104.83 = 0.09 ±0.28. This effect was due to the interaction between coat colour and sex; among the black goats the females had a significantly higher temperature while among the white the males were significantly higher, both at the five per cent point. The mean figures were as follows:—

	Black	*White*
Female	105.7	104.0
Male	105.1	104.7

The mean of five animals had each a Standard Error of ±0.28.

As regards the body temperature rise on sun exposure, black goats showed a significantly greater rise (at one per cent point) than white. Difference between values for ten black goats and for ten white goats was 2.94—1.87 = 1.07 ±0.36. In this respect there was no significant difference between the sex means (10) for males, 2.11 and females, 2.70, the difference, 0.59, having S.E. ±0.36. Considering the effect of body weight, there was absolutely no correlation between body weight and (i) initial body temperature (r = —0.0350) or (ii) maximum rise on sun exposure (r = —0.0296).

In regard to the cooling effect of the shower the following means were obtained :—

Colour	*Sex*	*Initial temp.*	*Min. temp.*	*Fall*
Black	F	102.82	101.46	1.38
	M	103.14	101.02	2.12
White	F	102.34	100.96	1.38
	M	102.56	100.46	2.10
All colours and sexes		102.72 ±0.15	100.98 ±0.23	1.745 ±0.20

When considering the initial body temperature, the minimum body temperature reached and the fall in body temperature on exposure

to shower, there was no significant difference between colour and sex. The means were as follows :—

	Initial temp.	*Min. temp.*	*Max. fall*
Black	102.98	101.24	1.75
White	102.45	100.71	1.74
Males	102.85	100.74	2.11
Females	102.58	101.21	1.38
S.E. of means	±0.21	±0.33	±0.28

As regards the effect of body weight on the temperatures recorded during the shower there was a negative correlation which approached significance, r = —0.4188 (the five per cent point is r = ±0.4683). There was thus some evidence that a higher body weight was associated with a smaller fall of body temperature on exposure to shower.

Effect of coat colour in sheep. On May 9th, 1946 at Izatnagar twelve black and twelve white sheep were tethered four feet apart in a well-screened area in the sun, black and white animals alternating. Exposure was from 1-30 to 4-30 p.m. (dry bulb temperature 105 to 106 to 104° F) and the body temperature readings were taken every 30 minutes. The wind velocity was only 1.4 m.p.h. The two groups were practically identical.

	Black	*White*
Bodyweight (lb.)	41.1	41.1
Height (in.)	22	21.7
Heart girth (in.)	23.8	24.0
Length (in.)	21.8	22.2
Hb (g. per cent)	7.8	8.0

All the sheep were yearlings except one two-year-old, and all were males except two females, one in each group. Most of the white animals had some brown or black markings about the head while some of the black ones had a few spots of white on some part of the body. The white animals were slightly poorer in condition than the black. All had been clipped the day before the experiment and were bled for haemoglobin estimations two days after experiment. Water was withheld during the exposure, after which the animals drank well. The mean maximum temperature recorded for the white animals was 105.1°F and for the black 106.1°F, the difference being significant. The mean maximum rise for all animals after exposure was 2.22 ±0.16 which is significantly different from zero. There was a significant difference (one per cent point) between the means of the black and of the white sheep, 2.78—1.66 = 1.12 ±0.31.

The effect of coat colour and of removing the coat in sheep. At Mukteswar on May 30th, 1947, ten black sheep and ten white ones were exposed in a sheltered yard to sun, black and white being placed alternately.

The following day they were clipped and on June 1st were given a second exposure for about three hours from 9 a.m., body temperature readings being taken every 30 minutes. The weather conditions on the two days differed more than was desired, the dry bulb temperature ranging from 72 to 82°F on the first and from 79 to 87°F on the second. The mean values were as follows :—

	Black	*White*
Body weight after clipping (lb.)	59.2 (42-74)	40.4 (32-47)
Height (in.)	22.9	22.2
Heart girth (in.)	28.1	25.1
Length (in.)	23.7	21.5

Unfortunately animals of similar age were not available and thus of the large sheep four were from three years eight months to seven years four months and the remainder from one year five months to two years three months. All except one were females two of which were pregnant. Of the small sheep one was three years and the remainder from one year to one year ten months; two were females, five were entire males and three were castrates. The following mean values for body temperature were obtained :

	Pre-exposure	*Mean max. temp.*	*Mean max. rise*
Black sheep (unclipped)	102.18	104.00	1.82
„ „ (clipped)	102.10	105.04	2.94
White sheep (unclipped)	102.35	104.06	1.71
„ „ (clipped)	102.50	105.17	2.67
All animals	102.28	104.57 ±0.02	2.28 ±0.12

There were no significant differences in the initial body temperatures of the black and white animals or of unclipped and clipped. As to the maximum temperature obtained on exposure, there were no significant differences between the means for coat colour, the black animals reaching 104.52°F and the white 104.62°F. There was, however, a highly significant difference (one per cent) between the means for the unclipped and for the clipped, viz. 104.03°F and 105.10°F, a difference of 1.07 ±0.13. Unfortunately the air temperature to which the clipped ones were exposed was several degrees higher than that which obtained for the unclipped. This fact invalidates the conclusion that the wool acts as a protection against heat as it does against cold. The same is true of the maximum rise and is particularly true of the black coloured animals. Within the white group there was significantly more variation in the readings than with the black. As to the maximum rise in temperature after exposure, there was again no significant difference between the means for coat colour, both black and white being 2.19. There was again, however, a highly significant difference (0.1 per cent) between the means for the unclipped and for the clipped, viz. 1.76 and 2.80, a difference of 1.04 ±0.24.

Again, as for coat colour, with the unclipped sheep analysed as a separate group, there was no significant difference between the means for coat colours which were 102.18°F for the black and 102.35°F for the white animals; S.E. 0.15. The same was true of a group of ten animals, the corresponding figures being black 102.10°F and white 102.50°F; S.E. 0.19. When the data in relation to coat colours and maximum rise of body temperature on exposure were similarly treated there was a similar lack of significance.

	Black	*White*	*S.E.*
Unclipped	1.82	1.71	±0.24
Clipped	2.94	2.67	±0.32

Respiration rates taken on June 1 were 149 per minute for the black and 28 for the white, and many of the black sheep panted on both days. Had the atmospheric temperature on this day been somewhat hotter the rectal temperatures of the black sheep might have risen significantly higher than the white ones. As it was, the variances of the two groups were heterogeneous so that the individual means had to be converted into ordinary logarithms before analysis. The mean logarithmic rate of the black was then found to be significantly greater than that of the white animals. The wool clipped from every animal was weighed, the means being 9.8 ounces for the black and 11.4 ounces for the white: the difference was not significant.

Effect of body size in goats. In an attempt to probe further the question whether for heat tolerance goats with large bodies are better adapted than those with small ones, twenty white goats (ten heavy and ten light) were held in a sheltered yard from 11 a.m. to 3 p.m. at Mukteswar on May 22nd, 1945, body temperatures being taken hourly. Air temperature rose from 70 to 75°F. Large and small goats were placed alternately and their positions occasionally moved so as to assist uniform heating. Five were from 2½ to 7 years, the remainder being 13 months or less. Body weights ranged from 52 to 97 lb. for the older animals and from 18.7 to 49 lb. for the younger. Body height/weight ratio was from 0.31 to 0.44 for the older and 0.40 to 0.95 for the younger. All were females save two (one yearling and one seven-year-old) and four were lactating. A few goats in both the large and small groups had coats with faint brown or reddish splashings and of the two groups the coats of the smaller were a purer white.

The values obtained for means of ten animals were as follows:—

	Light	*Heavy*	*S.E.* (+)	*Diff.*	*S.E. of diff.* (+)
Initial body temp.	102.06	101.86	0.19	0.20	0.25
Max. temp. on exposure	104.29	103.74	0.23	0.55	0.32
Max. rise in body temp.	2.23	1.88	0.20	0.35	0.28

When the ten heavier animals are compared in respect of body weight with the ten lighter ones none of these differences is significant nor by the correlation co-efficient method could anything significant be found between body temperature and initial temperature, maximum temperature or maximum rise in temperature, values for r being respectively —0.360, —0.422 and —0.153.

Any disadvantage which the smaller goats might have suffered from over heating, even had it proved significant, could not have been attributed to body size for it might have been an age effect or both.

A repetition of the previous goat experiment, using ten large and ten small goats, was carried out at Mukteswar on May 29th, 1947. Selection was made so that coat colour in the two groups corresponded as closely as possible, being wholly black or black with a minimum of white or tan, or various shades of brown. Six of the large goats and seven of the small were black or nearly so. Mean values of body measurements were as follows :—

	Large	*Small*
Bodyweights (grand mean of 3 weighings) (lb.)	58.4(47–80)	33.7(24–43)
Height (in.)	24.6	20.1
Heart girth (in.)	27.7	22.4
Length (in.)	24.3	20.7

Of the large goats five were from two years two months to three years old, two were over three years and three were nine to sixteen months; eight were females and two castrated males. Of the small ones four were nine to twelve months and the remainder from three to five months, half being castrated males and half females. The animals were tethered in a sheltered place in the sun, large and small alternately, from 9 a.m. to 11-30 p.m. and rectal temperatures recorded every half hour. Dry bulb temperature ranged from 74 to 82°F. The resulting means and their S.E.'s are tabulated below:—

	All animals (20)	*Light* (10)	*Heavy* (10)	*Difference*	*Significance level of difference*
Initial body temp.	102.20 ± 0.12	102.50 ± 0.17	101.89 ± 0.17	0.61 ± 0.24	5%
Max. body temp.	104.48 ± 0.20	105.11 ± 0.28	103.86 ± 0.28	1.25 ± 0.39	1%
Max. rise	2.29 ± 0.21	2.61 ± 0.30	1.97 ± 0.30	0.64 ± 0.42	—

Comparison between body weight and the items in question by the correlation coefficient method were as follows :—

Initial body temp. r = —0.398 not significant.
Maximum body temp. r = —0.603 highly significant.
Maximum rise r = —0.406 not significant.*

*Level of r required for significance at 5 per cent = ±0.444; at 1 per cent = ±0.561.

Effect of body size and coat colour in goats. A further experiment to compare the heat resistance of large and small white goats and large black goats was carried out at Izatnagar on May 13th, 1946. There were twelve animals in each category and they were exposed in a screened area of 468 square yards from 12-40 to 4-5 p.m., body temperatures being recorded every 30 minutes. Air temperatures were 99 to 100.5 to 99°F and wind velocity at body level averaged 1.1 m.p.h. The following are details of the animals :—

	Large white	*Small white*	*Large black*
Body weight (lb.)	34.5—45.5(m.40.1)	19.6—36(m.26.5)	28.8—43(m.35.5)
Heart girth (in.)	21.5—25 (m.23.4)	19—21.5(m.20.2)	21—24 (m.22.5)
Hb (g. per cent)	6.7— 8.1(m. 7.3)	6.1—7.6(m. 6.8)	6.1—7.9(m. 7.0)
	8M 4F	7M 5F	4M 8F

In the large white group there were five aged 1 to 1½ years, six from 2 to 2½ years and one of three years. The small white group comprised ten at 1 year and two at 1½ years; and the large black group six at 1 to 1½ years and six at 2 to 2½ years. The general condition of all the animals was rather poor. Some of the white goats had a little black or brown about the head. Blood was taken for haemglobin estimations on May 14th and May 16th. Means for the data obtained were as follows :—

Category	*Initial temp.*	*Max. temp.*	*Rise*
Large white	103.03 ±0.33	104.79 ±0.28	1.76 ±0.30
Small white	104.20 „	105.58 „	1.38 „
Large black	103.30 „	105.37 „	2.07 „
All animals	103.51 ±0.19	105.24 ±0.16	1.73 ±0.12

The significance of these differences is as follows. With the initial body temperature the small white is significantly higher than the large white at the five per cent point but not quite significantly higher than the large black. There is no significant difference between the mean maximum temperature attained in the three groups. The large black gave a significantly higher rise than the smaller white (five per cent).

From May 17th to May 23rd the temperatures of the same animals at rest in their shed were taken daily at 8 a.m. and 4 p.m. During these seven days the dry bulb temperature ranged from 72 to 92° F at 8 to 9 a.m. and 79 to 98°F at 4 to 5 p.m. In the large black group one animal, and in the small white group two, had to be excluded because they showed abnormally high temperatures throughout, due presumably to some temporary indisposition. With these exceptions the mean body temperatures were as follows :—

	Large white	*Small white*	*Large black*
8 a.m.	102.1	102.8	102.1
4 p.m.	102.7	103.6	102.9

The difference between the morning and evening temperatures in each of the three categories of animals was highly significant. With the morning temperatures the differences between the small white and large white and between the small white and large black were also highly significant. Similarly with the evening temperatures, the same two differences were highly significant. It is clear therefore that the normal temperatures of small goats are somewhat higher than those of larger ones, an effect which may be concerned both with age and body size.

DISCUSSION

Findlay (1950) has emphasised the meagre knowledge available on heat regulation in goats, especially in respect to different breeds. In considering the points brought out in this paper, reliance has been placed on three body temperature effects; the initial body temperature, the mean maximum (or minimum) temperature level, and the mean maximum rise (or fall) in temperature of animals exposed to various climatic conditions. The first effect to be considered is that of the coat colour of sheep and goats on body temperature when the animals are exposed to warm or hot sun. It is well known that white, and especially smooth white, coats reflect much of the sun's rays which would otherwise be absorbed by the body and so embarrass heat regulation. The woolly nature of the fleece of sheep might do much to prevent this heat reflecting effect in animals of this species (Lee and Phillips, 1948).

In one experiment a group of small white goats gave significantly higher temperatures (5 per cent) than a group of large white goats, while a group of large black goats gave a significantly higher maximum rise (5 per cent) than the small white goats.

Another matter for enquiry concerns the influence of body size on exposure to hot sun. A general hypothesis might be that a light body, with little musculature and a relatively large surface area, would have a higher body temperature and be more affected by exposure to sun, than a heavy body. This was supported by experiments described in the paper, especially that on the comparison of 10 light and 10 heavy goats, with variously shaded coats, in which the mean maximum temperature of the light animals after exposure was greater than that of the heavy ones at the 1 per cent level of significance. In all the other experiments in which the weight of animals was taken into consideration a negative relationship was found to exist between body weight and the maximum body temperature reached on exposure to sun though the resulting correlation coefficients were not large enough to be significant except in the case of the above-mentioned experiments on goats. This property of heavier animals being better able to withstand the effect of sun heat may be attributable to advantage in chest capacity, age, or physical condition.

When exposed to a shower of water instead of to the sun, there is a fall in body temperature, the extent of which seems to be unaffected either by sex or coat colour. With the goats in two experiments the mean maximum falls in body temperature following a shower are significant. The extent of body temperature fall tends to be greater with lighter animals and, although in neither case did the negative correlation coefficients for body weight with temperature drop reach significance level, the results lend some support to the hypothesis that lighter animals are less able to withstand fluctuations in climatic conditions.

The above findings concern goats. When observations were made on sheep, all of a nondescript variety, the results are less conclusive. The work reported by Lee and Robinson (1941) suggests that sheep are relatively tolerant to hot atmospheres. In one experiment reported in this paper no significant differences could be found between animals of dark or light coat colour, or heavy or light bodyweight, in respect of either rise or fall in body temperature following exposure to sun or shower respectively. In one experiment however, the body temperature of black sheep showed a significantly greater rise on exposure to sun than that of white sheep, and in another the mean rise of clipped sheep was very significantly greater than of unclipped sheep, though in this case the difference in coat colour was not significant.

In regard to the statistical analysis, it is possible that insufficient numbers of animals were employed. Although groups of 10 or so were used in some experiments this was not always adequate to prove a particular point. For instance, in the experiment in which it was desired to compare three large buffaloes with three small ones, the substitution of another large buffalo for one that proved to be a defaulter might have led to a definite conclusion. In practice, of course, that would have meant including all large buffaloes available

CONCLUSIONS

Experiments are described showing the effects of body cooling and body overheating in sheep, buffaloes and goats.

The results have been analysed statistically.

In clipped sheep, body temperature followed a lower course for a time, but after twenty days rose to that of unclipped animals. Both buffaloes and goats exposed to strong winds showed lower temperatures than sheltered groups.

Temperatures were significantly higher in buffaloes standing in hot sun and irrespective of giving or withholding water or of wetting the body surface, than in those kept in the shade.

In goats, heavy animals withstood the effect of the sun's heat better than light ones, and when exposed to cooling influences the fall in body temperature tended to be greater in light animals. The

results were also affected by coat colour. The fall in temperature induced by cooling, however, seemed to be unaffected by coat colour.

In sheep, the results were less conclusive, suggesting that they were relatively tolerant to hot atmospheres except that black sheep showed a significantly greater temperature rise on exposure to sun than white sheep.

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SOME FACTORS INFLUENCING THE SEASONAL VARIATION IN WORM EGG COUNTS IN SCOTTISH HILL SHEEP

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INTRODUCTION

Morgan and co-workers (Morgan and Sloan, 1947; Morgan, Parnell and Rayski, 1950), have shown that the seasonal output of helminth eggs in the faeces of Scottish hill sheep follows a cyclic pattern, with minimal values in January and peak values in May or early June. Further work (Morgan, Parnell and Rayski, 1951; Wilson, Morgan, Parnell and Rayski, 1953; Parnell, Rayski, Dunn and Mackintosh, 1954) indicates that this "Spring rise" in worm egg output is paralleled by an increase in worm burden, this being due mainly to an increase in worms of the Trichostrongylidae.

Since this seasonal pattern of egg output has an important bearing on problems of helminth control in hill sheep, it was thought desirable to present in somewhat greater detail than hitherto, the effects of certain factors on this cycle of seasonal variation as shown by an analysis of data collected by Morgan and his co-workers on two Scottish hill farms where the work was carried on for several years.

The factors chosen for present consideration are :

(*a*) the influence of the season of the year on the form of distribution of worm egg counts between sheep, using the finding of Hunter and Quenouille (1952) that the parameters of the negative binomial satisfactorily define distributions of worm egg counts of naturally infested sheep, (*b*) the effect of weather, in particular the relative severity of the winter months, on the pattern of worm egg output, and (*c*) the average changes in worm egg output associated with increasing age of sheep. As the sheep were numbered the present data allow for the examination of the effect of age over several seasons.

In addition to average changes in resistance with age, the variations in egg output, as shown by individual sheep from year to year, have also been studied. It is of practical importance to know if sheep which show abnormally high worm egg counts as lambs, or hoggs, retain a tendency to high counts when adult.

MATERIAL

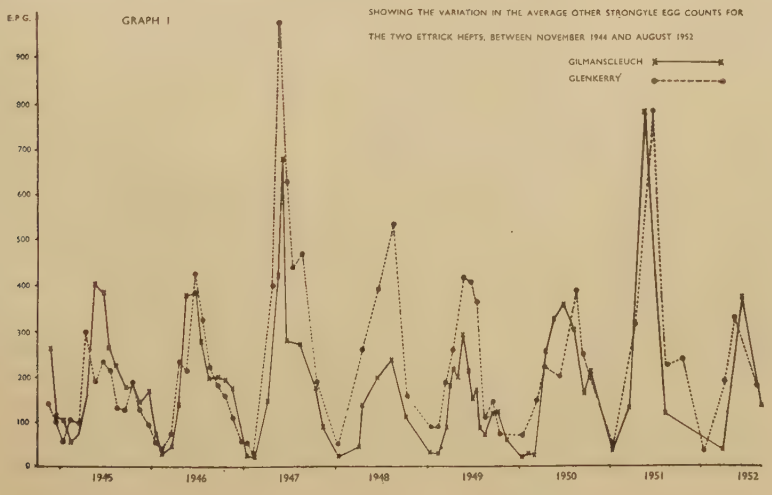
The data used are the worm egg counts of Scottish Blackface sheep on the Hope Heft of Gilmanscleuch and on the Fullcleuch Heft of Glenkerry; the former is in Ettrick, the latter in the tributary Tima Valley. Both were farmed by the late Mr. A. Linton. On these hefts continuous observations on the seasonal variations in worm egg output were started in November, 1944. (Morgan and Sloan, 1947; Morgan, *et. al.*, 1951). During the first four years, November 1944 to 1948, both hefts were sampled at monthly intervals; between 1948 and 1952 sampling was continued at less regular intervals.

All the sheep were numbered and no anthelmintic treatments were used on these hefts during the period of investigation. The egg counting technique used is detailed by Morgan, *et. al.* (1950) whereby the eggs are grouped under three categories—"Strongyloides", "Nematodirus" and "Other Strongyles". Consideration has been limited to eggs of the latter category, numerically and clinically the most important. Worm egg counts were made on four cells of the McMaster slide and the totals multiplied by 50 to give the number of eggs per gram of faeces. Analyses have been carried out on the totals as found i.e. without multiplication by 50.

RESULTS

Seasonal Variation in Counts of "Other Strongyle" Eggs

The variations in the mean counts of "Other Strongyle" eggs in the faeces of the sheep on the two hefts sampled during the period November 1944 to October 1952 are shown in Graph 1. The mean counts shown are derived from all sheep on the two hefts, amounting to some sixty to seventy sheep per heft at each sampling. In order to maintain the numbers of sheep approximately constant through-



out the year, counts from the lambs are included and counts from the draft ewes excluded in September of each year.

The cyclic pattern of seasonal variation in average egg output is similar in both hefts, with minimal average values of usually less than 100 eggs per g. of faeces in January, increasing to an average value of some 500 eggs per g. at the peaks in May or June. Years 1947 and 1951 are characterised by spring peaks of worm egg output which are approximately double those occurring in other years.

Examination of the distribution of counts for differing seasons and in differing years, with calculation of the negative binomial parameters k and p (Hunter and Quenouille 1952), amplifies the picture of mean variation by giving, in addition, an estimate of the seasonal changes in dispersion of eggs in the flock. To estimate k,

TABLE 1

CALCULATED VALUES OF P AND K FOR THE TWO FLOCKS SAMPLED

NOVEMBER 1944 — OCTOBER 1947

Fullcleuch Heft, Glenkerry					*Hope Heft, Gilmanscleuch*				
Date of sampling	*No. of sheep*	*Mean count*	*p*	*k*	*Date of sampling*	*No. of sheep*	*Mean count*	*p*	*k*
Nov. 1944	64	2.77	9.23	0.30	Nov. 1944	61	5.34	11.87	0.45
Dec. ,,	63	1.95	5.57	0.35	Dec. ,,	64	2.36	5.24	0.45
Jan. 1945	58	1.10	4.23	0.26	Jan. 1945	53	2.02	8.42	0.24
Feb. ,,	60	2.13	7.10	0.30	Feb. ,,	55	1.16	6.10	0.19
March ,,	64	1.89	4.97	0.38	March ,,	60	1.48	7.40	0.20
April ,,	65	5.93	7.52	0.79	April ,,	53	3.26	10.52	0.31
May ,,	26	3.77	1.75	2.15	May ,,	52	8.10	10.80	0.75
June ,,	60	4.63	4.77	0.97	June ,,	58	7.64	8.68	0.88
July ,,	62	4.24	5.23	0.81	July ,,	61	5.13	5.13	1.00
August ,,	61	2.54	6.20	0.41	August ,,	55	4.45	7.30	0.61
Sept. ,,	63	2.48	3.31	0.75	Sept. ,,	68	3.43	5.04	0.68
October ,,	70	3.73	6.09	0.57	October ,,	65	3.57	4.96	0.72
Nov. ,,	71	2.56	6.92	0.37	Nov. ,,	62	2.81	6.11	0.46
Dec. ,,	67	1.87	9.35	0.20	Dec. ,,	68	3.31	7.70	0.43
Jan. 1946	70	1.03	3.13	0.33	Jan. 1946	64	1.34	7.05	0.19
Feb. ,,	71	0.69	1.73	0.40	Feb. ,,	57	0.53	1.66	0.32
March ,,	68	1.46	3.73	0.39	March ,,	67	0.81	2.61	0.31
April ,,	70	4.59	7.64	0.60	April ,,	64	2.67	7.41	0.36
May ,,	38	4.13	6.08	0.68	May ,,	60	7.58	9.97	0.76
June ,,	70	9.44	13.68	0.69	June ,,	45	7.62	10.88	0.70
July ,,	66	6.41	10.68	0.60	July ,,	56	5.46	10.70	0.51
August ,,	68	4.40	6.28	0.70	August ,,	61	3.87	5.37	0.72
Sept. ,,	73	3.56	4.19	0.85	Sept. ,,	66	3.95	10.39	0.38
October ,,	73	3.16	5.02	0.63	October ,,	57	3.88	9.70	0.40
Nov. ,,	75	2.13	2.84	0.75	Nov. ,,	55	3.44	6.61	0.52
Dec. ,,	72	1.35	2.69	0.50	Dec. ,,	58	1.16	2.64	0.44
Jan. 1947	71	1.03	3.43	0.30	Jan. 1947	57	0.42	1.50	0.28
Feb. ,,	75	0.51	3.64	0.14	Jan.(31st) ,,	59	0.46	0.38	1.20
April ,,	72	8.03	9.56	0.84	Mar.(31st) ,,	53	2.91	2.75	1.06
May ,,	30	19.63	16.50	1.19	May (9th) ,,	28	8.46	12.82	0.66
June ,,	69	12.55	7.65	1.64	May (27th) ,,	55	13.58	14.00	0.97
July ,,	69	8.74	4.05	0.84	June ,,	57	5.54	3.96	1.40
August ,,	69	9.33	7.71	1.21	August ,,	56	4.93	10.26	0.52
October ,,	73	3.74	5.27	0.71	October ,,	67	3.49	5.82	0.60

the rapid but reasonably efficient method of equating $\left(1+\frac{\bar{x}}{k}\right)^{-k}$ the expected frequency of zero counts, to the proportion of zero counts actually observed (Anscombe, 1949), has been used. Where $k\bar{x}$ does not exceed 4 this method is more than 75 per cent. efficient (Hunter and Quenouille, 1952). Data for p and k for all counts made during the experimental period are too extensive to present in full, but the degree of variation in p and k which may occur over a period, is illustrated by the data shown in Table 1. This covers the period November 1944 to October 1947, where the first two years show counts of normal magnitude at the spring peak, while in 1947 the spring peak appears to be exceptionally high.

Table 1 indicates that k, which may be regarded as an index of dispersion of egg output in the flock, changes from relatively low levels, usually less than 0.4, in the November to March period, to levels of usually greater than 0.6 in the period April to October. Within each of these periods k remains relatively constant, but shows occasional high values during the spring months; for example at Glenkerry k = 2.15 for May, 1945 and 3.0 for May, 1949. These high values of k are not directly associated with peak values of mean count, nor do the two years with high mean counts at the spring rise (1947 and 1951) show exceptionally high values of k at this time.

In general it would appear that during the spring and summer months the dispersion of worm eggs is more uniform than during the winter, with a maximum evenness of dispersion in May and June. This indicates that the spring rise of mean worm egg count involves most of the flock and is not due to exceptionally high counts in a few sheep at this time. Comparison of the two hefts studied shows that both behave in a broadly similar manner with respect to variation of k; no consistent difference in behaviour between the hefts could be noted. The variation in p is not markedly cyclic although in some years low values occur in the winter months when the level of the worm egg output is small.

To illustrate the fit of negative binomial distributions with exponents k, derived as given above, the observed and theoretical frequencies of the worm egg counts in January, May and June, 1949, at Glenkerry are shown in Table 2. The three counts shown cover a wide range of k and may be regarded as typical examples of counts made at other times. In view of the small numbers of sheep per unit, the agreement between observed and theoretical frequencies is reasonably satisfactory (P = .16 – .26). In general, a similar measure of agreement was found for other counts.

Since worm egg distributions take the form of negative binomial distributions, the question of a suitable transformation arose, so that valid variance ratio and t tests could be carried out. Hunter

TABLE 2

OBSERVED AND THEORETICAL FREQUENCIES

Other Strongyle Egg Counts on the Glenkerry Heft in January, May and June, 1949.

January				*May*					*June*				
Count	*Obs.*		*Exp.*	*Count*	*Obs.*		*Exp.*		*Count*	*Obs.*		*Exp.*	
0	44		43.42	0	1		1.25		0	11		11.00	
1	13		10.43	1	4	11	2.77	8.10	1	8		8.01	
2	4		5.73	2	6		4.08		2	8		6.57	
3	3		3.74	3	11		5.00		3	6		5.58	
						11		10.51			16		10.41
4	1	6	2.63	4	0		5.51		4	10		4.83	
5	2		1.93	5	5		5.68		5	6		4.22	
						14		11.25					
6	3			6	9		5.57		6	3	10	3.72	11.23
7	1			7	3		5.27		7	1		3.29	
						8		10.12					
10	2	8		8	5		4.85		8	4		2.92	
11	1		7.12	9	3		4.36		9	4		2.60	
27	1			10	1		3.85		10	0		2.32	
						12		14.43			11		16.29
	75		75.00	11	3		3.35		11	2		2.08	
				12	5		2.87		12	0		1.86	
				13	2				13	0		1.67	
				14	1				14	0		1.50	
				15	1				15	1		1.34	
				17	2				16	2			
				19	1	12		13.59	17	2			
				20	1				18	1			
				28	1				19	1			
				30	2				23	1			
				33	1				25	1	12		12.49
									27	1			
									31	1			
									63	1			
									106	1			
					68		68			76		76	
$k = 0.28$ $x = 1.70$				$k = 3.00$ $\bar{x} = 8.35$					$k = 0.80$ $\bar{x} = 8.16$				
$\chi^2 = 1.909$				$x = 2.772$					$\chi^2 = 5.182$				
$p = .17$				$p = .26$					$p = 0.16$				

and Quenouille (1952) suggest the use of the Beall (1942) transformation of $y = K \text{ Sin h} - 1 \left(1 + \frac{\bar{x}}{k}\right)^{-k}$ with k taken as equal to 0.7, but because of the variability of k, we initially preferred the use of the arbitary transformation of $y = \log_{10}(1+x)$, but most of the analysis has also been carried through using the former transformations. That the two transformations appear to be approximately equivalent in their effect, is seen from the following listed values.

Count	*Score**	*Log* (1+x)	"*Difference*"
1	0.85	0.30	0.55
2	1.08	0.48	0.60
3	1.23	0.60	0.63
5	1.43	0.78	0.65
10	1.71	1.04	0.67
30	2.16	1.49	0.67
50	2.37	1.70	0.67
70	2.51	1.85	0.66
100	2.66	2.00	0.66

(*Score given by Hunter and Quenouille for transformation of worm egg counts.)
Based on $y = K \operatorname{Sin} h\left(\frac{1+\bar{x}}{\bar{k}}\right)^{-k}$

The "difference" between the two scores remains approximately constant over a wide range of count, suggesting that the change of scale achieved by either transformation will be very similar.

Analysis of Seasonal Variation

Analysis of seasonal variation shown in Graph 1 has been limited to counts made in October, January, April, June and August in each year. June counts were taken in preference to May counts, although in some years the latter show higher values, since difficulty of gathering during or soon after lambing usually reduced the number of sheep sampled in May. Weighted means for counts made in these months are shown in Table 3.

Analysis of variance using both the "Beall" and the logarithmic transformations showed the following variance ratios :

Glenkerry	*Degrees of Freedom*	*F. (Beall)*	*F. Log.*(1+*x*)	*Significance*
Due to				
Years	7	9.22	9.11	x x x
Months	4	145.38	128.44	x x x
Interaction	28	5.17	5.33	x x x
Error (Mean Square)	2721	(0.396)	(0.164)	
October	7	6.18	4.35	x x x
January	7	1.25	0.75	N.S.
April	7	4.58	4.14	x x x
June	7	5.94	9.65	x x x
August	7	11.96	11.53	x x x
Gilmanscleuch				
Years	6	6.72	8.26	x x x
Months	4	128.21	131.37	x x x
Interaction	24	5.02	4.76	x x x
Error (Mean Square)	2094	(0.398)	(0.146)	
October	6	1.84	1.53	N.S.
January	6	1.70	1.50	N.S.
April	6	7.97	7.73	x x x
June	6	9.87	11.03	x x x
August	6	5.36	5.49	x x x

In this paper x x x shows significance at 0.1 per cent. and x at 5.0 per cent.

TABLE 3

AVERAGE "OTHER STRONGYLE" EGG COUNTS (EGGS/G. FAECES)

In the period November 1944 - August 1952

Glenkerry

Year	*October*	*January*	*April*	*June*	*August*	*Mean*
1944-45	(64) 138	(58) 55	(65) 297	(60) 231	(61) 127	(308) 172
1945-46	(70) 186	(70) 51	(70) 229	(70) 422	(68) 219	(348) 221
1946-47	(73) 158	(71) 51	(72) 401	(69) 627	(69) 466	(354) 337
1947-48	(73) 187	(75) 50	(72) 258	(73) 388	(73) 537	(336) 283
1948-49	(69) 154	(75) 84	(76) 255	(76) 408	(72) 105	(368) 203
1949-50	(75) 70	(73) 68	(68) 218	(70) 397	(72) 389	(358) 226
1950-51	(70) 195	(74) 43	(72) 312	(49) 787	(64) 223	(329) 280
1951-52	(66) 241	(73) 43	(71) 196	(62) 333	(58) 186	(330) 195
Mean	(560) 165	(569) 56	(566) 271	(529) 440	(537) 288	(2761) 241

Gilmanscleuch

Year	*October*	*January*	*Late March Early April*	*Late May Early June*	*August*	*Mean*
1944-45	(61) 267	(53) 101	(53) 163	(52) 404	(55) 223	(274) 232
1945-46	(65) 178	(64) 67	(64) 134	(60) 379	(61) 193	(314) 188
1946-47	(57) 194	(57) 21	(53) 145	(55) 679	(56) 267	(278) 260
1947-48	(67) 175	(58) 23	(61) 45	(60) 194	(61) 239	(307) 137
1948-49	(69) 110	(69) 27	(62) 181	(58) 292	(67) 66	(325) 129
1949-50	(64) 119	(62) 21	(67) 27	(61) 325	(63) 315	(317) 159
1950-51	(67) 205	(68) 32	(70) 159	(52) 781	(57) 116	(314) 237
1951-*	No count	No count	(73) 31	(66) 378	(63) 136	
Mean	(450) 177	(431) 41	(430) 120	(398) 427	(420) 201	(2129) 189

Numbers of sheep sampled shown in brackets.
*Year 1952 omitted from analysis at Gilmanscleuch.

The effect of the season of the year, the difference between years, and the month/year interaction, were all significant at the 0.1 per cent. level.

With regard to the 'between year' variation for individual months, January worm egg counts were not significantly different at either heft. The significant differences in April and June can be ascribed at Glenkerry to the high counts in the two years 1947 and 1951. Counts in these two years were significantly greater

(0.1 per cent. level) than those of other years which do not differ significantly among themselves. At Gilmanscleuch counts made in these two years were also significantly greater than the rest (1 per cent. level in April and 0.1 per cent. level in June). The following August and October counts for these two years—1947 and 1951—were not exceptional on either heft: thus there appears to be a rapid recovery from the high worm burden responsible for the high counts in June. Year 1948 was characterised by the lateness of the spring rise; maximal values were reached in the summer in contrast to other years where the peak was in June.

Effect of Weather

The exceptional counts in June 1947 and 1951 appear to be associated with the severity of the immediately preceding winters. The relative severity of the winter for the period under review can be judged from Table 4 where the degree of departure of mean monthly temperatures from the normal, as determined at Eskdalemuir, is shown. The low temperatures experienced in the winters of 1947 and 1951 seem to be the main factor responsible for the high worm egg counts in June of these years. The negative correlation between average temperatures deficit shown in Table 4 and the June counts was significant for both hefts. Temperature figures alone are not enough to assess fully the severity of a winter, particu-

TABLE 4

DEPARTURES OF MEAN MONTHLY TEMPERATURES FROM THE NORMAL (°F)

Compared with June Counts

	Nov.	*Dec.*	*Jan.*	*Feb.*	*March*	*April*	*Average*	*June counts (eggs/g. of faeces)* Glenkerry	Gilmanscleuch
1944-45	—2.3	—0.1	—5.4	+4.2	+4.9	+2.7	+0.67	231	404
1945-46	+2.4	+1.0	—2.8	+2.1	—0.2	+3.1	+0.93	422	379
1946-47	+2.4	—1.8	—2.1	—9.1	—5.5	0.0	—2.68	627	679
1947-48	—0.6	+1.6	—0.1	+0.6	+5.0	+2.2	+1.45	388	194
1948-49	+1.0	+1.4	+2.2	+2.4	+0.7	+3.7	+1.90	408	292
1949-50	+0.4	+1.5	+1.0	—0.7	+3.6	—0.9	+0.82	397	325
1950-51	—2.6	—5.4	—1.1	—1.8	—3.4	—2.4	—2.78	787	781
1951-52	—3.5	+0.1	—5.6	—1.0	+2.0	+3.2	—0.80	333	378

Analysis

	Regression Coefficient	*Significance*
Gilmanscleuch ..	101.5	$N_1 + N_2 = 1 + 6, F = 38.2$ x x x
Glenkerry	73.4	$N_1 + N_2 = 1 + 6, F = 8.3$ x

larly as it affects sheep on the hill. Driving rain, snowstorms and the length of periods with snow lying are probably of greater importance. However, in Ettrick, a district of high rainfall, with the lowest ground above the 700 ft. contour, there is probably a close association between a long period of low temperatures and a surface of frozen snow. In such severe winters hill sheep may exist under conditions of semi-starvation and show excessive loss of body weight during the winter months. For example. the live weight figures of hoggs during the winter and spring 1950-51, as indicated in the table, show that between October and April there can be a loss of body weight of hoggs of between 15 per cent and 25 per cent. (Table 5). It is of interest that minimum body weight is reached about April, shortly before the time of the maximum "O.S." egg counts. Improvement in condition after April is rapid and is followed by the sharp decrease in worm egg output after the spring peak. The weight changes of the older sheep during winter and spring are complicated by the effect of pregnancy; usually some loss of weight is evident in gimmers and ewes, but the effect is not so pronounced as with the hoggs.

TABLE 5

AVERAGE WEIGHT (LB.) AND STANDARD ERRORS DURING 1950-51 WINTER FOR HOGGS

		Oct. 25th 1950	*Jan. 24th* 1951	*Apr. 4th* 1951	*June 13th* 1951	*Aug. 7th* 1951
Glenkerry	Av. Wt.	62.1	56.3	46.3	60.6	74.5
	St. Error	±2.02	±1.43	±1.02	±1.56	±1.71
		Oct. 20th 1950	Jan. 9th 1951	Mar. 27th 1951	May 24th 1951	Aug. 14th 1951
Gilmanscleuch	Av. Wt.	64.0	62.9	55.4	64.6	79.4
	St. Error	±1.52	±1.35	±1.40	±1.93	±2.40

Data for rainfall and for hours of sunshine are given in Table 6. The rainfall during the bad winters of 1946—47 and 1950—51 was not excessive, and in general there is no apparent association between amounts of rainfall during winter months and worm egg counts at the spring rise. The same appears true for the total amounts of sunshine. In the year 1948, when the rise in worm egg count was delayed and peak values were during the summer, instead of in May-June, weather conditions were near normal, except that March especially and May were warmer and drier than normal, April was average but June was exceptionally wet (220 per cent. of the normal) and sunless. Adverse conditions during June may account for the high August counts, the good weather of the previous months, March especially, being determining factors in the low counts in the June period.

TABLE 6

RAINFALL AND SUNSHINE RECORDS FOR NOVEMBER 1944-52
Eskdalemuir Observatory

Year	*Total Rainfall (in.)*		*Total Sunshine (hours)*	
	Nov. - April	*May - Oct.*	*Nov. - April*	*May - Oct.*
1944-45	40.58	30.31	380.0	762.1
1945-46	22.95	28.90	458.3	750.9
1946-47	40.75	18.84	312.8	782.4
1947-48	37.10	35.31	453.9	754.5
1948-49	35.57	21.16	435.4	935.6
1949-50	42.72	38.38	383.7	718.6
1950-51	30.42	23.92	422.7	824.9
1951-52	36.05	22.72	434.9	763.8

Age of Sheep and Worm Egg Count.

Using the series of worm egg counts made in successive years it is possible to collect together those pertaining to any one particular age; thus for the age of six months there are October worm egg counts of sheep born in the previous spring made in the years 1945 to 1951 inclusive. At the other extreme, i.e. seventy-six months of age there are the August worm egg counts made in the period 1945 to 1952 inclusive for animals born in the years 1939 to 1946. Worm egg counts for intermediate ages are collected in a similar manner. This age grouping of counts made over eight years reduces the effect of exceptional seasons but it would require a much longer period to average out entirely the effect of weather.

A selection of average "O.S." egg counts classified according to age are shown in Table 7, the numbers of sheep sampled per group being shown in brackets. The marginal means of age give an estimate of the changes in egg output which are due to age of sheep *per se.*, uncomplicated by seasonal effects. These necessarily cover yearly periods as Scottish hill lambs are born in April or May, and variations due to age found with a unit of one year cannot therefore be divorced from seasonal effects.

The distributions of counts for any particular age are in the form of negative binomial distributions. Analysis of variance of transformed counts (Beall) gives the following values for significance :

TABLE 7

AVERAGE COUNTS (EGGS/G. OF FAECES)

Other Strongyle Eggs according to Age

Heft		*Months of count*					
Fullcleuch, Glenkerry	*Age (months)*	*October*	*January*	*April*	*June*	*August*	*Mean*
	6–16	(94) 355	(106) 186	(102) 589	(81) 459	(101) 524	420
	18–28	(87) 253	(101) 54	(96) 261	(85) 541	(94) 306	275
	30–40	(81) 119	(93) 28	(101) 229	(77) 400	(92) 314	214
	42–52	(73) 91	(90) 8	(93) 109	(79) 377	(87) 174	148
	54–64	(83) 73	(90) 22	(86) 173	(75) 370	(86) 169	155
	66–76	(78) 64	(90) 15	(76) 162	(70) 435	(79) 175	160
	Mean	167	56	263	432	286	235
Hope, Gilmans-cleuch		*October*	*January*	*Early April*	*June*	*August*	*Mean*
	6–16	(70) 267	(81) 141	(93) 213	(75) 432	(95) 411	293
	18–28	(64) 321	(68) 26	(94) 143	(75) 408	(92) 269	232
	30–40	(74) 165	(72) 19	(86) 75	(67) 338	(75) 140	142
	42–52	(59) 88	(67) 9	(75) 69	(61) 185	(74) 79	84
	54-64	(57) 56	(72) 13	(78) 83	(66) 236	(79) 96	96
	66–76	(64) 54	(71) 12	(73) 77	(61) 243	(69) 89	92
	Mean	163	39	114	314	194	162

Number of sheep in brackets.

Glenkerry			
Due to	*Degrees of Freedom*	*F.*	*Significance*
Months	4	131.43	x x x
Age	5	59.66	x x x
Interaction	20	8.81	x x x
Error	2583	(0.3714)	
Subdivision of age			
Within 1st 3 years	2	33.51	x x x
Within 4th to 6th years	2	0.32	
Between 1st 3 and 4th—6th years	1	230.62	x x x
Gilmanscleuch			
Due to	*Degrees of Freedom*	*F.*	*Significance*
Months	4	123.46	x x x
Age	5	62.72	x x x
Interaction	20	3.27	x x x
Error	2177	(0.3424)	x x x
Subdivision of age			
Within 1st 3 years	2	31.10	x x x
Within 4th to 6th years	2	0.48	
Between 1st 3 and 4th—6th years	1	259.40	x x x

There is a steady fall in average worm egg output during the first four years of life after which the level remains constant. The significant interaction between age and months is an indication of the changes in seasonal behaviour with increasing age. For older ewes the rise and fall about the peak value in June is more acute than that for hoggs and gimmers; thus at Glenkerry, the ratio of worm egg counts of draft ewes to that of hoggs in April and August respectively is 1 : 3.63 and 1 : 2.99 while in June it is 1 : 1.06, and at Gilmanscleuch the April and August ratios are 1 : 2.69 and 1 : 4.33 compared with a June ratio of 1 : 1.78. Thus, with increase in age the duration of the "spring rise" decreases and in this connection it is of interest that the increase of resistance with age more than offsets the effects of pregnancy. The means at the foot of the table again show the effects of the season of the year, the low winter worm egg counts and the spring rise. Although the January worm egg counts are relatively low throughout they also show the increase in resistance with age.

Variation shown by Individual Sheep

Worm egg counts for individual sheep were summed to give yearly totals; comparison of these gives a measure of the influence of the apparent infestation as hoggs on subsequent worm egg output. As an example of the type of data, yearly totals for Gilmanscleuch sheep born in 1944, are shown in Table 8. Results for sheep born

TABLE 8

YEARLY TOTALS (OCT.—SEPT.) OF O.S. COUNTS

Individual sheep born 1944 Gilmanscleuch

Sheep No.	*Oct.* 1944–*Sept.* 1945	*Oct.* 1945–*Sept.* 1946	*Oct.* 1946–*Sept.* 1947	*Oct.* 1947–*Sept.* 1948	*Oct.* 1948–*Sept.* 1949	*Oct.* 1949–*Sept.* 1950
B1 ..	87	34	12	11	16	2
B2 ..	77	93	42	15	2	9
B3 ..	161	102	65	17	9	9
B4 ..	97	63	58	8	18	11
B5 ..	169	163	64	23	13	5
B6 ..	24	24	11	6	17	0
B7 ..	64	15	10	6	16	9
B58 ..	59	25	15	6	9	6
B9 ..	33	11	10	5	12	8
B10 ..	52	57	54	6	17	21
B11 ..	82	54	36	20	7	12
B12 ..	208	74	28	2	15	3
B13 ..	57	62	33	4	14	10
B16 ..	193	209	97	36	67	41

*To convert to eggs per g. of faeces multiply by 50.

Analysis

Significance of Regression

1st and 2nd years N_1 and N_2 = 1 + 12 F = 17.40 Significant at 1%
1st and 3rd years do. do. F = 8.51 Significant at 5%
1st and 4th years do. do. F = 5.05 Significant at 5%
1st and 5th years do. do. F = 2.59 Insignificant
1st and 6th years do. do. F = 1.19 Insignificant

in other years also show some measure of correlation between performance in the first year and in subsequent years. Thus at Gilmanscleuch for sheep born in 1945 and 1946, the regression of the second and third year counts on the counts of the first year are at least significant at the 5 per cent. level. Regression for counts in years subsequent to the third year are not significant. For sheep born in 1947 the regression is not significant but the abnormally severe spring of this year may have affected performance of the lambs. Again for sheep born in 1948 and 1949 the regression is significant at 1 per cent. and 5 per cent. for first and second years respectively, and at 5 per cent. for first and third years for the 1948 group.

Results for Glenkerry are very similar, except for sheep born in 1944, where there is no significant correlation between performance in the first and subsequent years. It would appear, therefore, that there is a significant tendency for sheep which show a high count in the first year, to show high counts in the following years. This trend is on the average significant at the 1 per cent level for the first and second years, at the 5 per cent. level for the first and third years, and not significant for the years subsequent to the third.

DISCUSSION

The correlation in the seasonal pattern of worm egg count and the worm burden (Morgan, *et al.*, 1951) in Scottish hill sheep, suggests that the variations found in worm egg count throughout the year are due primarily to seasonal changes in worm burden. With under three to six weeks as the time necessary for the completion of the prepatent period of the cycle, the spring peak of egg output would appear to be associated with larvae picked up in the February to April period. Climatic conditions at this time are far from optimal for the development of infective stages, and Crofton (1949) has shown that it is not until August that the peak numbers of infective larvae are found on hill pastures in Northern England. This fact lead Cushnie and White (1948) to the view that the spring rise is produced by worms carried over from infestation acquired during the previous autumn. However, the finding by Morgan, *et al.* (1951) and of Parnell, *et al.* (1954) of large numbers of young stages in March and April, in addition to the general rise in worm burden, indicates that the numbers of larvae ingested at this time are able to establish themselves in the host. Under hill conditions, therefore, the resistance of the host appears to be of greater importance than the availability of larvae; even under severe winter conditions, the intake of larvae appears sufficient to allow the development of heavy infestation. This view is in agreement with the increased rise observed after winters of exceptional severity such as in 1947 and 1951. The lowering of host resistance in such seasons, appears to more than offset the reduced incidence and

accessibility of infective free living stages consequent to severe climatic conditions. As judged from the present data, a severe winter results in high counts in sheep of all ages and is not limited to the hoggs although the latter are more susceptible. The hoggs also show relatively more weight loss. Low temperatures during winter and early spring appear to be of importance in determining the height of the spring rise; June counts show an average increase of 101 and 73 eggs per g. of faeces at Gilmanscleuch and Glenkerry respectively for an average monthly temperature deficit of 1°F during the preceding November to April period. Probably this effect is not directly due to cold *per se* but rather to increased nutritional deficiencies during cold spells. Decreased body vigour, associated with underfeeding and exposure in severe winters, appears therefore to be a primary factor in the development of an abnormal spring rise. Recent work by White and Cushnie (1952) has shown that in hoggs the worm egg count can be reduced and the spring rise almost prevented by the use of a simple dietary supplement, thus emphasising the importance of nutrition in the cycle of worm egg output. More exact data are required on the effect of supplementary feeding of hill sheep to determine those levels which give economic return in terms of meat, wool and of reduced helminthiasis.

The change in trend of egg output in May to July occurring at a time when climatic conditions are more favourable for the development of infective larvae, also indicates the subordinate role of pasture contamination on the level of parasitism attained with hill sheep. This sudden reversal in trend of egg output which terminates the spring rise, has many features in common with the phenomena of 'self cure' as observed by Australian workers and may be associated with the succession of species in Scottish hill sheep. Under Australian conditions 'self cure' appears to be closely related to a renewal of growth in herbage and indeed Gordon (1948) has suggested the possibility of 'anthelmintic' factors in rapidly growing grazing plants. More recent work on the mechanism of 'self cure' (Stewart 1950, 1953, and 1953b) indicates that the reaction occurs in sheep which are hypersensitive as a result of the intake of frequently spaced doses of larvae. It is likely that a reaction of this type is responsible for the fairly abrupt loss of infestation occurring in late spring in Scottish hill sheep. The fact that egg counts made 2 to 4 months after the abnormally high peak counts of the 'bad years' are no greater than those found for the same times in normal years, suggests that rapid elimination of worms can occur. However, because of the numbers of worm species involved and also because of the possibility of interactions between these species it would seem probable that the seasonal pattern of egg output results from a complex of factors of which resistance of the host is only one.

The changes in egg output with increasing age shown in Table 7 indicate the extent of the acquired resistance which occurs in hill

sheep under conditions of normal management without anthelmintic dosing. Hoggs appear to be some three times as susceptible to infestation as do older ewes, full resistance being developed after three years of age. Hoggs and gimmers, therefore, play an important role in the contamination of hill grazing, these two together giving rise to more eggs per annum than the four categories of ewes. It is possible that pasture contamination by hoggs, and to a less extent gimmers, during the winter and early spring months materially assists in the maintenance of the seasonal cycle of egg production by making available infective larvae at a time when flock resistance is minimal. The value of wintering hoggs away, or on the low ground, as a means of reducing hill pasture contamination is evident, but if they are being wintered on the hill, anthelmintic treatment in autumn, winter and spring, which is known to be of considerable direct benefit to hoggs, must reduce very considerably the numbers of larvae on the grazings and, therefore, perhaps, indirectly help the older sheep on the hill. A further aspect of resistance lies in the variability of performance of individual sheep. As seen from Table 8, sheep born on the same heft in the same year may show widely differing counts. Thus for the fourteen sheep born at Gilmanscleuch in 1944, the total count in eggs per g. of faeces in the period October 1944 to September 1945 covers the range of 1,200 to 10,400. That experimental sheep, receiving the same dose of infective larvae and subject to similar treatment, can show widely differing degrees of response, is well known (Gibson, 1954), and may be attributed to differences in inherent resistance. It is of interest that such differences are traceable up to some three years of age, suggesting the possibility of helminth control by selective breeding or by selective dosing of heavily infected lambs and hoggs

CONCLUSIONS

A statistical study is made of factors influencing the worm egg counts on two hefts of Scottish hill sheep during the period November 1944 to August 1952. The spring rise in count is shown to be highly significant and the degree of rise is associated with the severity of the preceding winter. Effect of age on worm egg count is highly significant. Hoggs of 6 to 16 months of age show counts some three times greater than ewes over the age of three years, and between 4 and 6 years of age has no significant effect on worm egg counts. Sheep born on the same heft in the same year may show widely differing counts and these individual differences persist up to the age of three years.

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THE OPTIMUM CONCENTRATION OF LAPINISED RINDERPEST VIRUS FOR THE INOCULATION OF RABBITS IN THE PRODUCTION OF VACCINE

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INTRODUCTION

The production of lapinised rinderpest virus vaccine requires large stocks of "bank" virus because rabbits are inoculated with 50,000 ID*50* doses of the virus. These stocks are cumbersome and, therefore, the effect of the inoculation of lower concentrations of virus was investigated.

MATERIALS AND METHOD

Rabbits of the Albino, Chinchilla and Rex breeds, $4\frac{1}{2}$ months old, were inoculated intravenously with 1.0 ml. of varying dilutions of dried infected tissues of known titre. Twenty rabbits were used in each experiment. Ten received 50,000 ID*50* doses of virus and the other ten had 10,000, 1,000, 100 or 10 ID*50* doses respectively. Temperatures were recorded daily until the rabbits were killed 65 hours after inoculation. The pathognomic lesions in rinderpest infection in the rabbit are in the tonsilla caecalis major, the sacculus rotundus, the appendix, the Peyer's patches and the spleen (Fukusho and Nakamura, 1940 ; Brotherston, 1951). They were assessed on a 4 plus basis, making a possible total of twenty points per rabbit. The spleens, mesenteric lymph glands and the blood were collected in the usual manner, emulsified, shell frozen and dried on a freeze-drier (Hale and Walker, 1946). Drying was completed in ampoules

TABLE 1

COMPARISONS OF LESIONS IN RABBITS INOCULATED WITH VARYING CONCENTRATIONS OF LAPINISED VIRUS

	Concentration of Virus Inoculated (ID50)			
	10	*100*	*1,000*	*10,000*
	7·9 ± 1·4	12·0 ± 3·2	16·6 ± 2·0	17·1 ± 1·7
Standard (50,000 ID50s) (inoculated)	17·7 ± 1·4	16·9 ± 2·2	15·8 ± 1·9	16·7 ± 1·7
F	10·51	2·16	1·14	2.16
t	—	2·832	0·661	0·506
P	<0.01	$<0.05>0.01$	>0.4	>0.05

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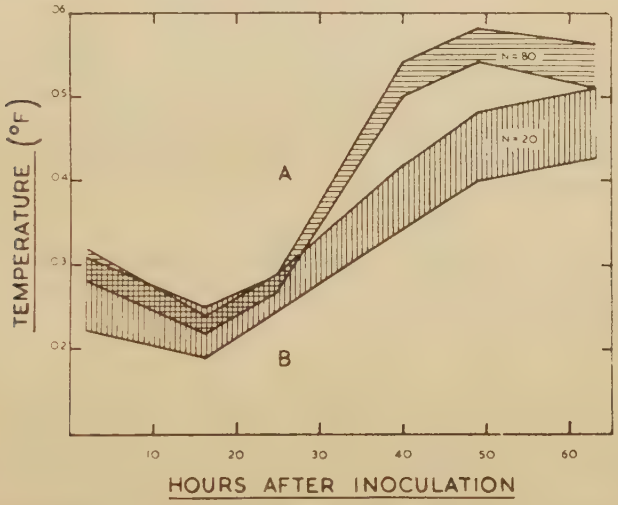
over phosphorous pentoxide and the ampoules were sealed under vacuum. The dried material was titrated in rabbits, using four animals for each dilution. The F and t tests of the Null Hypothesis were applied to each pair of values.

RESULTS

Data are shown in Fig. 1, Tables 1 and 2. When 1,000 ID*50* doses or more were inoculated the results, as measured by the temperature curves, the lesions and the titre of the injected material, were identical. The inoculation of 100 ID*50* doses resulted in a significantly

FIG. 1



The confidence limits of thermal reactions of rabbits inoculated with A> 1,000 ID*50* and B< 1,000 ID *50* lapinised rinderpest virus.

TABLE 2

COMPARISON OF VIRAL TITRES.

Expressed as the logarithm of the ID*50* dose, from rabbits inoculated with varying concentrations of lapinised virus.

	Concentration of Virus Inoculated (ID50s)			
	10	*100*	*1,000*	*10,000*
	3·0 ± 0·9	4·3 ± 2·0	5·0 ± 1·0	5·5 ± 0·0
Standard (50,000 ID*50*s) inoculated —	4·5 ± 1·3	4·8 ± 0·8	5·0 ± 1·0	5·3 ± 2·0
F	2·00	6·32	—	—
t	3,000	0·739	—	0·455
P	∠0·05 7 0·02	7 0·4	7 0·9	7 0·05

slower thermal response and less definite lesions at the time of harvest although the titre of the tissues was the same. The response to 10 ID*50* doses differed in all three respects resulting in a slower thermal reaction, poorer lesions and a lower titre.

DISCUSSION

The inoculation of 50,000 ID*50* doses of lapinised rinderpest virus in the production of lapinised rinderpest vaccine is unnecessary because the critical level of virus was found to be 1,000 ID*50* doses.

CONCLUSIONS

The response of rabbits to the inoculation of 1,000 ID*50* doses or more of lapinised rinderpest virus was identical. When fewer ID*50* doses were administered the thermal response was slower, the lesions less definite and the titres of the tissues lower.

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STUDIES ON *PASTEURELLA SEPTICA*

I. The Occurrence in the Nose and Tonsils of Dogs

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INTRODUCTION

Pasteurella septica and certain haemolytic, pasteurella-like coccobacilli have frequently been reported as being present in the respiratory tract of healthy animals. Table 1 summarizes a number of publications in which carrier rates for various animals have been recorded.

TABLE 1

INCIDENCE OF *PASTEURELLA SEPTICA* AND HAEMOLYTIC COCCOBACILLI IN THE RESPIRATORY TRACT OF HEALTHY ANIMALS ACCORDING TO VARIOUS AUTHORITIES

	Animal	*Site examined*	* *Carrier rate*	*Authority quoted*
Pasteurella septica	Buffalo	Nose	3·5% (7/200)	Singh (1948)
	Pig	Tonsil, mouth, lymph nodes	51%(95/188)	Meinicke (1932)
	Pig	Lungs	26% (13/50)	Lisac (1934)
	Cat	Air passages	75% (15/20)	Schenk (1938)
	Cat	Respiratory tract	90% —	Lenormant (1941)
	Rat	Throat	14% (14/102)	Schipper (1947)
	Rat	Throat	17% (31/188)	Hulphers and Henricson (1943)
Haemolytic coccobacilli	Ox	Tonsils	6% (6/100)	Francis (1941)
	Ox	Nose	8% (9/105)	Bosworth and Lovell (1944)
	Sheep	Nose	40% (40/100)	Bosworth and Lovell (1944)

Infections due to *Past. septica* or haemolytic coccobacilli occur in all the animals listed in Table 1 with the possible exception of the rat. Since pasteurella infections appear to be rare in the dog it seemed of interest to discover whether *Past. septica* or haemolytic coccobacilli occurred normally in the respiratory tract of this species, and if so, with what frequency. The opportunity to do this came when a survey was made of the incidence of a number of bacterial species in the nose and tonsils of dogs. The results as regards *Past. septica* are recorded herewith.

*The percentage carrier rate is given, followed in brackets by the number of positive cultures and the total number of animals examined.

MATERIAL AND METHODS

One hundred and eleven dogs destroyed at the Royal Veterinary College and Hospital, London, during the period 1950-53 for reasons other than disease were examined for *Past. septica*. Most of the dogs came from households in the London area and were destroyed at the owners' request for economic or similar reasons. Others were street accident cases in which the animal had been killed outright or destroyed shortly after the accident. All were apparently healthy. Nasal and tonsillar swabs were taken immediately after destruction by electrocution or by an intravenous overdose of nembutal. One swab was used for both nostrils and one for both tonsils and within one hour each swab was streaked on to a 5 per cent ox blood agar plate. After 24 hours incubation at 37°C. the plates were examined for colonies resembling *Past. septica*. Suspicious colonies were filmed and, after subculture, transferred to carbohydrate and other diagnostic media. Cultures were not recorded as *Past. septica* unless they were small, Gram-negative, non-motile, non-spore-forming rods or coccobacilli growing on ordinary media under aerobic conditions. Glucose, sucrose and usually a variety of other carbohydrate substances, though not salicin, were attacked with the production of acid but not gas. No change was produced in litmus milk and gelatin was not liquefied.

When examining the plates, not only was the presence or absence of *Past. septica* recorded but also in most cases an estimate was made of the percentage of colonies which were *Past. septica*. In this way some idea could be obtained of the degree of colonisation of the animal's mucous membranes by *Past. septica* whilst eliminating errors due to differences in the size of the inocula. From these observations three numerical expressions were obtained.

(1). Carrier rate. This was based on the percentage of subjects yielding *Past. septica* from the nose and tonsils respectively.

(2). Mean percentage plate count (Mean P.P.C.). The mean of the percentage counts of *Past. septica* colonies in all positive plate cultures. For example, in a group of 4 dogs whose tonsils yielded plate cultures in which *Past. septica* constituted 0, 10, 40 and 100 per cent respectively of the total colonies present, the mean tonsillar P.P.C. would be 50 per cent.

(3). Incidence of *Pasteurella septica* as dominant flora. This indicated the percentage of subjects in which *Past. septica* colonies outnumbered any other type of colony in the primary cultures from the nose and the tonsil respectively.

When the primary plate cultures were very crowded no estimate of the number of colonies present could be made. The values given in the tables for the mean percentage plate counts and incidence as dominant flora have therefore been compiled from observations on less than the total 111 dogs examined.

RESULTS

Haemolytic, pasteurella-like coccobacilli were not isolated from any of the dogs examined. *Past. septica* as defined above was isolated from the nose, tonsils or both nose and tonsils of 61 of the 111 dogs (55 per cent). It was far more frequent in the tonsils than in the nose; indeed in only two subjects was *Past. septica* isolated from the nose but not the tonsils. The data are given in Table 2.

TABLE 2

OCCURRENCE OF *PASTEURELLA SEPTICA* IN THE NOSE AND TONSILS OF DOGS DESTROYED FOR REASONS OTHER THAN DISEASE.

	Nose			*Tonsils*		
	Number			*Number*		
	positive	*examined*	%	*positive*	*examined*	%
Carrier rate	11	111	10	59	110	54
Incidence as dominant flora	6	89	7	36	88	41
Mean P.P.C.*		10	58		55	51

The tonsillar carrier rate was considerably higher than the nasal (54 per cent compared with 10 per cent). Nevertheless, when *Past. septica* occurred in the nose it was capable of no less heavy colonisation than in the tonsils (mean nasal and tonsillar P.P.C.s of 58 and 51 per cent respectively). *Past. septica* was frequently the dominant bacterium recovered from the tonsils and was in fact preponderant in the tonsil more often than any other bacterium, surpassing in this respect even the alpha-haemolytic streptococci. It was recovered from the tonsils of 45 per cent of the dogs examined.

There was little difference between dogs of different age groups as regards the occurrence of *Past septica.* Table 3 summarizes the results. In the case of the nose, the frequency of isolation and the purity of the cultures was highest in young adult dogs (1 to 3 years old). In dogs of 4 to 8 years of age *Past. septica* was less frequent and it was not isolated from any of the aged dogs (8 years and over) examined. The figures for the tonsil show no great variation between the different age groups.

In Table 4 the results are analysed according to the season of the year in which the dogs were examined. For this purpose the period May to September has been regarded as the warm season and the period October to April as the cold. The seasonal differences shown are slight. All three values for nasal isolation are higher for the winter months than for the summer, whilst the figures for the tonsil show insignificant variation.

*Mean percentage plate count; the figure given under "number examined" is the number of dogs from which this mean has been compiled.

TABLE 3

OCCURRENCE OF *PAST. SEPTICA:* VARIATIONS ACCORDING TO AGE.

	Nose			*Tonsils*		
	Number			*Number*		
	positive	*examined*	%	*positive*	*examined*	%
Carrier rate						
Dogs under 1 year	1	19	5	11	19	58
Dogs 1–3 years	5	29	17	18	29	62
Dogs 4–8 years	5	46	11	22	46	48
Dogs over 8 years	0	17	0	8	16	50
Incidence as dominant flora						
Dogs under 1 year	1	17	6	6	17	35
Dogs 1–3 years	3	22	14	10	23	44
Dogs 4–8 years	2	36	6	15	36	42
Dogs over 8 years	0	14	0	5	12	42
*Mean P.P.C.**						
Dogs under 1 year		1	38		11	47
Dogs 1–3 years		4	77		16	47
Dogs 4–8 years		5	47		21	55
Dogs over 8 years		0	—		7	52

*(See note Table 2).

TABLE 4

OCCURRENCE OF *PASTEURELLA SEPTICA:* SEASONAL VARIATIONS.

	Nose			*Tonsils*		
	Number			*Number*		
	positive	*examined*	%	*positive*	*examined*	%
Carrier rate						
Period May—September	3	48	6	25	48	6
Period October—April	8	63	13	34	62	55
Incidence as dominant flora						
Period May—September	1	48	2	14	48	29
Period October—April	6	63	10	22	62	36
*Mean P.P.C.**						
Period May—September		3	51		24	56
Period October—April		7	62		31	49

*(See note Table 2).

In Table 5 where the results for male and female dogs are compared, all values are somewhat higher in the case of males. This is to say that *Past. septica* was isolated more frequently and in purer culture from male dogs than from bitches, and it formed the dominant flora of both the nose and the tonsil more often in male dogs than in bitches. The differences are slightly greater for the tonsil than for the nose.

TABLE 5

OCCURRENCE OF *PASTEURELLA SEPTICA:* VARIATIONS ACCORDING TO SEX

	Nose			*Tonsils*		
	Number			*Number*		
	positive	*examined*	%	*positive*	*examined*	%
Carrier rate						
Males	8	75	11	44	75	59
Females	3	36	8	15	35	43
Incidence as dominant flora						
Males	5	75	7	25	74	36
Females	1	36	3	9	35	26
*Mean P.P.C.**						
Males		7	61		41	53
Females		3	52		14	44

*(See note Table 2).

The strains isolated during the course of the survey differed in certain respects from the familiar type of *Past. septica* cultivated from cases of haemorrhagic septicaemia. These differences, however, are sufficiently important to be reserved for a later paper in which the characteristics of strains from a variety of animals will be compared.

DISCUSSION

The results obtained show that *Past. septica* is present in the respiratory tract of a high proportion of dogs. The carrier rate, based on the nose and tonsils combined, was 55 per cent., a figure similar to that obtained by other workers for the pig, rather lower than that for the cat and considerably higher than those for the ox and rat. Hæmolytic pasteurella-like coccobacilli were not found in dogs and according to the literature seem to be confined to ruminant animals.

Despite the high tonsillar carrier rate for *Past. septica* dogs do not appear to suffer frequently from disease caused by this organism. It is occasionally isolated as a secondary invader in the pneumonia of canine distemper and sometimes occurs in pleurisy, pneumonia and peritonitis in young puppies. Stevenson and Hilbert (1937) described an outbreak of tonsillitis with a few cases of terminal pneumonia in kennelled dogs, from which *Past. septica* was obtained. The organism is frequently isolated in heavy culture from the throats of dogs suffering from tonsillitis but the data presented above suggest that such findings may not necessarily be of pathological significance. Apparently the equilibrium between the canine host and its parasite *Past. septica* is a stable one; the dog seems to be able to accommodate large numbers of the parasite in his throat and yet

withstand any further invasion of the tissues; the organism for its part seems to have developed a high degree of adaptation to a benignly parasitic existence for it is widespread and abundant in dogs though rarely causing disease.

Local infections due to *Past. septica* sometimes occur in human beings bitten or scratched by cats but despite the widespread incidence of the organism in dogs infections following dog bites are rare (Allott, Cruickshank, Cyrlas-Williams, Glass, Meyer, Straker and Tee, 1944; Brunsdon and Mallett, 1953). This lack of infection in man may, however, be accounted for by certain differences between strains from dogs and cats which will be described in a later paper.

The higher incidence of *Past. septica* in young adult dogs of 1 to 3 years of age may be associated with the greater incidence in this age group of non-fatal cases of virus infections involving the respiratory tract. Some of the young dogs examined might recently have recovered from distemper in which *Past. septica* played a part as secondary invader and persisted on the respiratory mucous membranes after recovery. The higher incidence of *Past. septica* in the nose during the winter months may also be connected with the epidemiology of canine distemper, a disease which is most prevalent during the colder half of the year, particularly in the late autumn and early spring. The seasonal variations in the nasal carrier rate of *Past. septica* in dogs resemble those recorded by Straker, Hill and Lovell (1939) for pneumococci in the human subject. These workers found that pneumococci tended to be present most frequently during cold, damp seasons and that this seasonal variation was more pronounced in the case of the nose than the nasopharynx.

Past. septica was found less frequently in the nose of dogs of higher age groups and was absent from the nose of dogs over eight years of age. This tendency seems to be part of a general simplification of the nasal and tonsillar flora which accompanies advancing age in the dog and is probably connected with senile changes in the mucous membranes and with the full development of immunity against certain organisms.

The greater incidence of *Past. septica* in male dogs is of interest since a similar difference has been noted between the male and female carrier rates of pneumococci, meningococci and *Hæmophilus influenzae* in the human subject (Straker, *et al.*, 1939). No explanation for these differences can be suggested. It is perhaps worthy of note however that the male dog frequently carries on the mucous membrane of his prepuce an organism which has received the name *Hæmophilus canis* (*Bacillus hæmoglobinophilus canis*, Friedberger, 1903) but which in many respects resembles *Past. septica* and might after careful examination be worthy of re-classification as a member of the genus Pasteurella or even as a variety of *Past. septica*.

This organism appears to be absent from female dogs, a fact which may in some way be connected with the reduced incidence of *Past. septica* in the nose and tonsils of female dogs.

There seems to be a close analogy between the status of *Past. septica* as a member of the normal respiratory flora of dogs and that of the pneumococcus in man. Each occurs with a high frequency in the upper respiratory tract of its peculiar host species, each occurs more frequently in the nasopharynx or tonsil than in the nose and both show similar variations according to season and sex. Despite this similarity between the oecology of the two organisms, pneumococci have never been recovered from dogs and *Past. septica* has only on rare occasions been isolated from the healthy or diseased respiratory tract of men.

CONCLUSIONS

Past. septica was frequently isolated from the tonsils of healthy dogs, and less commonly from the nose.

When present in the nose or tonsil *Past. septica* was usually present in large numbers and was frequently the dominant bacterium recovered from the tonsil.

The nasal incidence of *Past. septica* was greatest in young adult dogs and was higher during the winter than during the summer months. These facts may be related to the epidemiology of respiratory diseases in the dog including canine distemper.

Past. septica was commoner in male dogs than in females.

There is some similarity between the status of *Past. septica* in the nose and throat of dogs and that of the pneumococcus in the nose and throat of human beings.

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ASPERGILLOSIS IN FREELIVING WILD BIRDS

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INTRODUCTION

Records of mycoses have so far been rare in freeliving wild birds although it is recognised as a common condition in captive specimens. Davis and McClung (1940) described aspergillosis in herring gulls frequenting the mudflats in the vicinity of Boston harbour and a similar case was recorded in the Thayer gull by Cowan (1943). It has also been observed in the puffin in Cornwall by Motton (1945). During an investigation concerning the epidemiology of a virus infection, Dane (1948) mentioned the presence of *Aspergillus fumigatus* in the air sacs of two manx shearwaters taken on the island of Skomer near the Welsh coast. The same disease has been reported in the black grouse, capercailzie, and a variety of wild-fowl in Sweden by Hulphers, Lilleengen and Henrieson (1941), in the mallard by Zeliff (1943) and in Hungarian partridges by Landy (1943). Severe outbreaks in young wild pheasants in Berkshire have been described by McDiarmid (1952), and Rothschild and Clay (1952) briefly drew attention to the occurrence of mycoses in wild wood pigeons in this country. The present communication records further cases in wild birds examined in recent years.

METHODS

Collection of specimens. At the commencement of these investigations it was found that dead or dying wild birds were extremely difficult to obtain in a state fit for examination in the laboratory; the delivery of suitable specimens was therefore sporadic. Some woodpigeons, condemned as unfit for human food, were eventually obtained by arrangement with the Leadenhall and Central Markets, London, and the remainder were found on this estate, dead or unable to fly. Unfortunately, such data does not permit of an accurate assessment of the morbidity rate. The jackdaw was one of many taken in cage traps at Compton and the herring gulls were forwarded by the late Mr. S. J. Motton, F.R.C.V.S., of Penzance. All the cases described were encountered during the period 1943-1953.

General procedure for examination. When a specimen was obtained, macroscopic abnormalities were noted, and smears, sections and cultures prepared. In selected cases certain viscera were preserved as museum specimens. Cultures were identified either at this laboratory or at the Hatherly Laboratories, Exeter, by courtesy of Dr. G. C. Ainsworth. In some instances laboratory animals were inoculated to confirm the pathogenicity of the aspergilli.

RESULTS

During the last ten years eighteen cases of aspergillosis have been confirmed in freeliving wild birds, in addition to those already recorded as occurring in severe enzootics of pheasants (McDiarmid, 1952). Fourteen of these were found in woodpigeons, three in herring gulls and one in a jackdaw. The causal agent, with a single exception, was *Aspergillus fumigatus*. The recovery of *A. nidulans* from one woodpigeon is noteworthy as this appears to be the first occasion that this particular species has been incriminated as a pathogen in wild birds and because, according to Thom and Raper (1945), infection of the air passages is comparatively rare in this species. The macroscopic lesions in all the herring gulls were very similar, consisting mainly of characteristic plaques throughout the thoracic and abdominal viscera. Fig. 1 depicts lesions on the surface of the gizzard and adjacent small intestine of one of these specimens.

Rabbits inoculated intravenously with suspensions of cultures from these gulls died about eleven days after infection and post-mortem examination showed extensive lesions in the thoracic and abdominal viscera in which the aspergillus could readily be demonstrated microscopically.

The pathological picture in the jackdaw was similar to that in the herring gulls, whereas the lesions in the woodpigeons varied in character from typical concentric plaques to large irregular necrotic nodules which were most marked in the livers of some specimens (Fig. 2). Without a confirmatory bacteriological examination, this latter type of lesion could readily be confused with tuberculosis in this species. Involvement of the respiratory tract was not so obvious in woodpigeons as in the other two species and the principal lesions appeared always to be localised in the peritoneal cavity.

DISCUSSION

The investigation of disease in freeliving wild animals presents many difficulties as the acquisition of specimens is a procedure which in many instances cannot be planned and depends on support given by gamekeepers and amateur naturalists. The predators which abound in nature rapidly dispose of sick or dead animals and consequently the cases obtained represent merely a fraction of the total number affected. Confirmation of the existence of a particular infection in a wild bird, serves at the most only to indicate the existence of a particular pathogen and the susceptibility of the various species. It is perhaps significant that the cases of aspergillosis confirmed in wild birds, either at Compton or elsewhere, have so far been found chiefly either in waterfowl or in species particularly fond of grain. The former have every opportunity to acquire mycotic infection from decomposing vegetable matter in the vicinity of streams, ponds or on the sea-shore, especially after a period of low tides, whereas the latter no doubt frequent heaps of fermentable material such as

discarded weed seeds and damaged bales of straw from combine harvesting operations. All the cases of mycosis in the vicinity of Compton have been encountered since the introduction of new farming methods in this area, although many pigeons were examined previously; perhaps the scarcity of references in the past to this disease in wildlife may be attributable in part to such changing conditions. The practice of placing in covert, uncovered heaps of weed seeds, which eventually become impregnated with masses of mycelium, may have a serious effect on pheasants and sometimes leads to heavy mortality before the source of the infection is realized. In pheasants the lesions are usually widespread throughout the viscera and follow the pattern of those in the herring gull; the kidneys are often severely affected (Fig. 3). Woodpigeons and jackdaws frequenting those heaps have the same opportunity to acquire infection as pheasants, although the latter, by the nature of their feeding habits, probably tend to disturb more spores. There can be little doubt that the general mortality in wild birds due to mycotic infection contracted in this way may be considerable, although largely unnoticed.

During the last few years many pigeons have been shot on this estate with a view to determining the incidence of infection with *Mycobacterium tuberculosis avium* and although from year to year, 2 to 4 per cent of these birds are found to have lesions of tuberculosis, no evidence of concurrent mycosis has so far been encountered in any of them. Conversely, tuberculous infection has never been found in any bird affected with aspergillosis.

CONCLUSIONS

Eighteen cases of aspergillosis have been confirmed in free-living wild birds.

The species affected were the Herring gull, (*Larus argentatus*), 3 ; jackdaw, (*Corvus monedula spermologus*), 1 ; and woodpigeon, (*Columba palumbus*), 14.

With the one exception of *A. nidulans* in a woodpigeon, the causal agent was *A. fumigatus*.

The lesions in all cases were extensive, involving most of the viscera.

FIG. 1. Herring gull (a) Plaques on the gizzard; (b) plaques on adjacent small intestine.

FIG. 2. Woodpigeon. (a) Large necrotic lesions in the liver; (b) lungs ; (c) pericardium.

FIG. 3. Pheasant. (a) Plaques in peritoneal cavity; (b) Kidney lesions.

FIG. 1

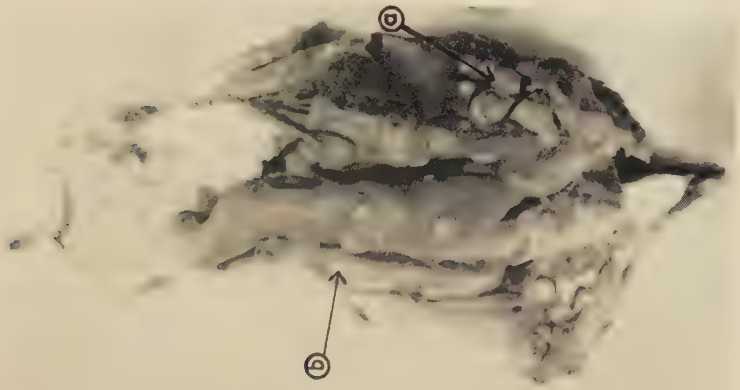


FIG. 2

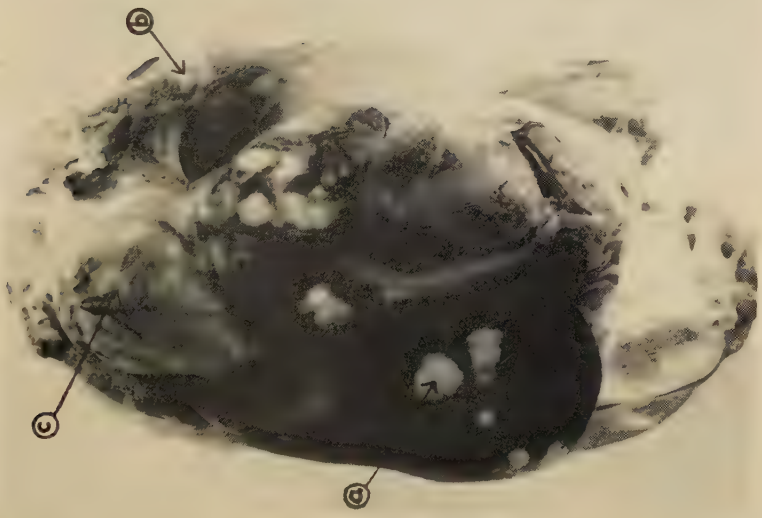
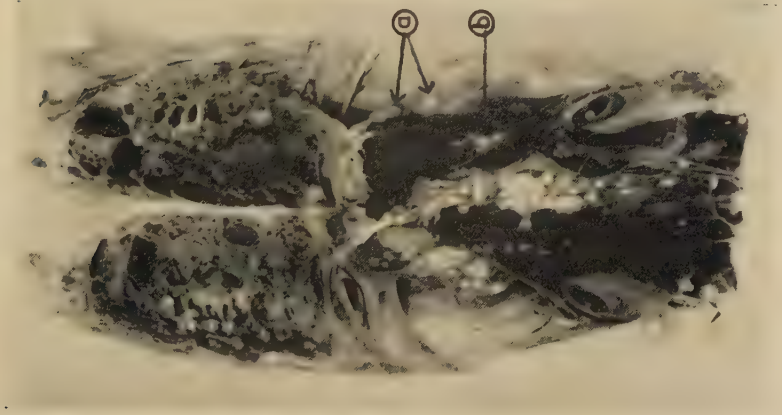


FIG. 3



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ACKNOWLEDGMENT

I am indebted to Mr. F. Summerfield, senior experimental officer at this Field Station, for preparing the photographs.

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THE ISOLATION OF *SALMONELLA GALLINARUM* IN WILD BIRDS

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INTRODUCTION

Biester and Schwarte (1948) quoting various authorities state that fowl typhoid occurs in a number of species of wild birds including pheasants, peacocks, swans, sparrows, wood pigeons, ostriches and quail. Jennings (1954) has reported the isolation of *Salm. gallinarum* from a blackbird and a curlew. Since 1947 there has been an increasing incidence of fowl typhoid in poultry in the East and North Ridings of Yorkshire (Sellers and Harbourne, 1953) and many outbreaks have occurred on isolated farms. The possibility that wild fauna might be concerned in the spread of the disease was suggested by farmers and other observers. Attention was drawn to the large numbers of rooks in the affected areas and their habit of feeding with poultry. Those interested were invited to submit for examination carcases of these and other birds which they had killed. In all, 42 rooks, 9 pigeons and 1 partridge were received from the East and North Ridings and 37 rooks from the West Riding.

METHODS

At post mortem examination direct cultures from the liver were made on nutrient agar. In addition, material from the same site and also the intestinal contents and in some cases the heart blood, were incubated in either tetrathionate or selenite broth for 18 hours. These enrichment cultures were plated out on bile salts, citrate agar (Difco S.S. agar) and after incubation, non-lactose fermenting colonies were seeded on to nutrient agar slopes and identified biochemically and serologically. On some occasions, feet and feathers from the birds were treated in a similar manner.

RESULTS

Natural Infection

Rooks (Corvus frugilegus frugilegus). 38 rooks were shot in the East Riding and 4 in the North Riding. *Salm. gallinarum* was isolated from 5 of the former and from 2 of the latter. All isolations were made from the enrichment cultures. 37 rooks killed in the West Riding, where the incidence of fowl typhoid is insignificant, were examined with negative results.

Salm. gallinarum was not isolated from the feet and feather cultures, but in the case of one batch of 6 rooks, *Salm. thompson* was recovered.

Wood pigeons (*Columba palumbus palumbus*). *Salm. gallinarum* was isolated from 1 of 9 wood pigeons shot in the East Riding.

Partridges (*Perdix perdix perdix*). 1 partridge, caught alive but obviously sick, was received and on post mortem examination showed lesions typical of sub-acute fowl typhoid, namely a bronzed liver, enlarged spleen and congested lungs. *Salm. gallinarum* was isolated by both direct and indirect cultural examinations. These results are summarised in Table 1.

TABLE 1

SITES OF ISOLATION OF *SALM. GALLINARUM* FROM WILD BIRDS

Species	*No. examined*	*No. positive*	*Sites of isolation*		
			Duodenum	*Liver*	*Heart Blood*
Rooks E. & N.R.	42	7	5	3	1
Rooks W.R.	37	0	0	0	0
Pigeons E.R.	9	1	1	1	1
Partridge E.R.	1	1	1	1	0

Other birds examined included jackdaws (8), plovers (1) and moorhens (1). All results were negative.

Experimental Infection

The extent of the experimental observations was limited by the number of wild birds which could be captured alive in areas of Yorkshire free from fowl typhoid. Unfortunately only 4 rooks were obtained but parallel experiments were performed on 13 jackdaws (*Corvus monedula spermologus*). These birds are easier to trap and zoologically are closely related to rooks and it was considered that infection of this species would differ little from that of rooks. Both the mode of administration and the material used to produce infection varied. Four birds were fed liver obtained from hens which had died of acute fowl typhoid. This was fed in two feeds on successive days. The other birds were infected by oral and parenteral inoculations with measured amounts of 18 hour nutrient broth cultures of recently isolated strains of *Salm. gallinarum*, containing approximately 800 million viable organisms per millilitre. Dead birds were examined using the methods previously described and in some cases the bile and droppings were treated similarly. The results are summarised in Table 2.

The post mortem appearance of the 11 birds which died and from which *Salm. gallinarum* was isolated was that seen in an acute infectious disease; the vent feathers were soiled and the liver, spleen and kidneys were enlarged and congested. The intestines showed a catarrhal inflammation, but in all cases the lungs appeared normal.

TABLE 2

EXPERIMENTAL INFECTION OF ROOKS AND JACKDAWS

Species	*Material*	*Dose*	*Survival (days)*	Salm. gallinarum *isolations*				
				H.B.	*Liver*	*Intest.*	*Bile*	*Droppings*
Rook	Infected liver	Fed on 2 successive days	D 3	+	+	—	—	—
,,	,,	,,	D 7	+	+	+	—	+
,,	,,	,,	K 57	—	—	—	—	—
Jackdaw	,,	,,	D 66	—	—	—	—	—
Rook	Broth culture oral	1 ml.	D 7	—	+	+	—	—
Jackdaw	,,	,,	K 57	—	—	—	—	—
Jackdaw	Broth culture s.c.	2 ml.	D 7	+	+	—	—	—
,,	,,	,,	D 57	—	—	—	—	—
,,	,,	1 ml.	D 6	+	+	+	—	—
,,	,,	,,	D 6	+	+	—	—	—
,,	,,	0.5 ml.	D 8	—	+	+	—	—
,,	,,	,,	K 91	—	—	—	—	—
,,	,,	0.25 ml.	D 10	+	+	+	+	—
,,	,,	,,	K 91	—	—	—	—	—
Jackdaw	Broth culture i.p.	1 ml.	D 5	+	+	+	—	—
,,	,,	0.5 ml.	D 6	+	+	+	—	—
,,	,,	0.25 ml.	D 6	+	+	+	—	—

K — Killed
D — Died

s.c. — subcutaneously
i.p. — intraperitoneally

The jackdaw which received 1 ml. of a broth culture by intraperitoneal injection had grossly inflamed intestines with blood in the lumen. *Salm. gallinarum* was not isolated from the remaining six birds. The jackdaw fed on infected liver died 66 days after infection and one of the jackdaws inoculated subcutaneously with 2 ml. of a broth culture died 57 days afterwards. Both birds were in a poor condition and there were no obvious pathological changes. The other four birds were killed.

Occasional blood tests were performed on these birds using the rapid whole blood plate test with a *Salm. pullorum* stained antigen. Three gave consistently positive results, one was positive until the 42nd day after infection and one, which gave a negative reaction on the 83rd day, was positive on the 91st day. The jackdaw fed on infected liver was tested once only, on the 48th day; the result was negative.

DISCUSSION

From the small number examined it is not possible to assess the significance of wild birds in the spread of fowl typhoid in poultry.

Nevertheless it has been established that the rook, the pigeon and the partridge can harbour *Salm. gallinarum*. All three species are common in the East and North Ridings of Yorkshire and particularly the rook, which is ubiquitous and is frequently seen feeding with poultry on free range.

Salm. gallinarum was isolated only from birds shot or killed in the East and North Ridings, where fowl typhoid is rife, and not from those obtained from the West Riding where the incidence is insignificant. This suggests that the part they might play in the spread of the disease is purely a local one.

Both rooks and jackdaws were susceptible to artificial infection and agglutinins were demonstrated in five of the survivors although *Salm. gallinarum* was not isolated from them. The longest period in which the organism was recovered was the tenth day after artificial infection and that in a bird which had died from the disease. The conclusion is that the artificially infected birds either died from an acute Salmonella infection or lived without becoming carriers of the disease. It is not known whether such conditions apply in the field. Nevertheless, mechanical transmission as opposed to actual infection must always be borne in mind. In three of the shot rooks *Salm. gallinarum* was isolated from the intestines only. It is feasible that they were merely receptacles of *Salm. gallinarum* as was undoubtedly the case with the rooks, where *Salm. thompson* was isolated from the feet and feathers. They may have been shot however before the organism had had time to penetrate the intestinal barrier.

Although it is by no means certain that rooks or other wild birds help to spread fowl typhoid, it is apparent that they can harbour *Salm. gallinarum*. Consequently any attempt at eradication in poultry based either on treatment or on blood testing may not be successful. However, if wild birds are temporarily infected only and contract this infection from poultry, elimination of the organism from domestic birds would be followed by its disappearance in wild birds also. An accurate establishment of this point would do much to explain the epizootiology of fowl typhoid.

CONCLUSIONS

Salm. gallinarum, the organism responsible for fowl typhoid, was recovered from seven rooks and one pigeon killed in areas of Yorkshire where the disease was enzootic. *Salm. thompson* was recovered from the feet and feathers of one rook. Fowl typhoid was diagnosed in one partridge. Thirteen jackdaws and four rooks were infected artificially with *Salm. gallinarum* by various routes and eleven of them died from an acute infectious disease. The possibility that rooks and other wild birds might spread the disease amongst poultry is discussed.

ACKNOWLEDGMENTS

Thanks are due to Dr. Joan Taylor of the Salmonella Reference Laboratory, Colindale, for confirming the identification of *Salm. gallinarum* from two rooks and for identifying *Salm. thompson*.

In addition I wish to thank my two colleagues, Mr. W. B. V. Sinclair and Dr. K. C. Sellers; the former for inoculating the experimental birds and the latter for his encouragement and valuable advice and I am also indebted to him for his criticism of the paper.

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THE COAGULATION MECHANISM OF THE BLOOD OF THE HORSE WITH PARTICULAR REFERENCE TO ITS "HAEMOPHILIOID" STATUS

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INTRODUCTION

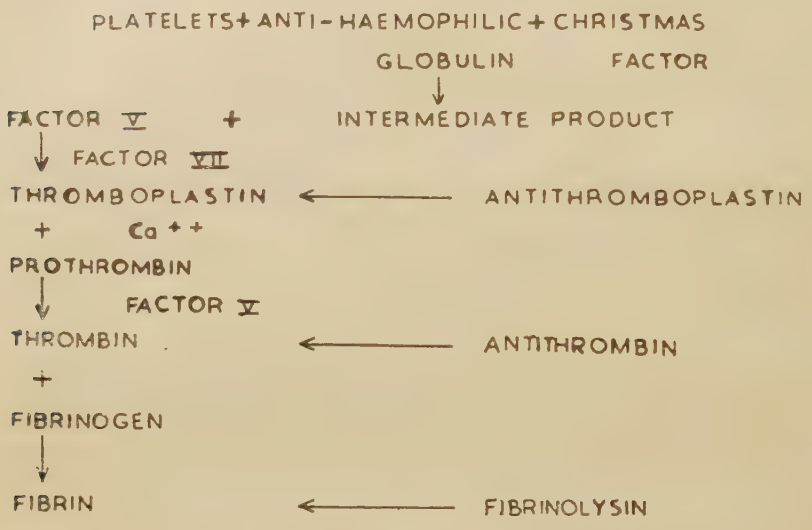
During the past decade rapid advances have been made in our knowledge of the coagulation mechanism. The blood of many vertebrates has been investigated with the aim both of increasing knowledge of the mechanism itself and of establishing suitable criteria for the use of animal blood as a laboratory implement for investigational work in the human subject. The animals most commonly studied have been the ox, dog, cow, rabbit and rat. In view of the large amounts of blood which may be safely withdrawn it is surprising that the horse has attracted remarkably little attention. Possibly, the rarity of spontaneous haemorrhage in the horse (Miller, 1954) accounts for this neglect. For these reasons, we were interested in measuring the coagulation factors of the horse in terms of known human values.

The coagulation mechanism, in the present state of knowledge, is regarded as consisting essentially of two reactions: the activation of prothrombin to thrombin and the interaction of thrombin with fibrinogen to produce fibrin, the basic "mesh" of the blood clot. Since the time when these processes were suggested by Morawitz (1904), knowledge has been broadened by the demonstration of many accelerating and inhibiting factors which influence these reactions. At present, the two principal accelerators are regarded as thromboplastin and Factor V (Ac globulin) while the chief inhibitors appear to be naturally occurring antithromboplastins, antithrombins and fibrinolysins. A generally accepted diagram of these interactions is shown in Fig. 1. It has now become apparent that thromboplastin formation itself is a very complicated process in which at least five components interact. The exact methods of these interactions are not definitely known but the suggested schema appears likely. If an "equilibrium" concept of the coagulation mechanism in the living body is accepted, a haemorrhagic state will result from a deficiency of prothrombin or fibrinogen, a deficiency of any of the accelerators or an excess of an inhibitor. Investigations in man bear out his concept. A thrombotic diathesis should,

theoretically, result from the opposite changes, but the present state of knowledge is much vaguer in this respect.

FIGURE 1

SIMPLE SCHEMA OF THE COAGULATION MECHANISM



METHODS

Test	*Method*	*Norman Human Values*
Platelet count	Rees-Ecker (1923)	200,000-400,000/cmm.
Clot retraction	Budtz-Olsen (1951)	over 45 per cent
1-stage prothrombin	Quick (1939)	80 to 100 per cent
Factor V	Stefanini (1950)	80 to 100 per cent
Factor VII	Koller, Lolliger and Duckert (1951)	80 to 100 per cent
Clotting time	Modified Lee-White (Ham, 1950)	glass 5 to 10 mins. silicone 18 to 25 mins.
Thromboplastin generation test	Biggs and Douglas (1953)	10 ± 1 sec. (100 per cent)
Protamine titration test for heparin	Allen and Jacobson (1947)	100-140 gamma protamine

RESULTS

Twelve horses of various types were carefully chosen so that haematological diseases were excluded as far as possible. A range

of ages, sexes and breeds was sampled. In addition to the coagulation tests, red and white cell counts were also made. The results are outlined in Table 1. The significant findings were the high red and white cell counts as compared with man, the slightly reduced platelet counts (except for the Shetland ponies), the prolonged one-stage prothrombin times with somewhat reduced Factor VII levels in some of the horses and relatively high Factor V assays and the very poor thromboplastin generation.

In man, a prolonged clotting time may be due to haemophilia (probably a lack of anti-haemophilic globulin), Christmas disease (deficiency of a β_2 globulin necessary for thromboplastin generation), a fibrinogenaemia, excess of a circulating anticoagulant, e.g. heparin, and in certain cases a qualitative platelet defect. The thromboplastin generation test may be used to differentiate most of these conditions. Biggs, Douglas and Macfarlane (1953) have shown that if aluminized plasma (prothrombin-free), serum, platelets and calcium are mixed together, a powerful thromboplastic activity develops. If this activity does not appear, substitution of normal fractions for the unknown will allow detection of the responsible component. In all tests, a standard human brain extract was used as a substitute for the platelets (Bell and Alton, 1954) to eliminate the platelet factor. Six of the horses were selected to include ranges of sex, breed and age. In all cases, the alumina plasma and serum yielded a very poor thromboplastin generation. Figure 2 shows the results of further tests performed in horse 4. Normal human serum did not increase the extent of generation, thus eliminating Christmas factor and Factor VII as the responsible factors. Biggs and Macfarlane (1953) have shown that as little as 10 per cent of the normal human Factor VII level will yield normal thromboplastin generation and the direct Factor VII levels performed in these horses were all considerably above this level. In contrast to human serum, normal human alumina plasma fully corrected the defect in all six instances. Therefore, it appeared that the horse lacks adequate anti-haemophilic globulin and/or Factor V to produce thromboplastin generation. Table 1 shows that the Factor V level in the horse is higher than in the human. In order to confirm the deficiency of anti-haemophilic globulin in horse plasma, alumina plasma from a human haemophiliac with a normal Factor V level was substituted for the horse plasma. No significant thromboplastin generation occurred.

DISCUSSION

Weiser (1922) and Hikmet (1927) have reported platelet counts in the horse ranging from 249 to 560,000/c.mm. Our results suggest a lower value except in the Shetland ponies. The degree of clot retraction manifested by these horses was very poor in view of the relatively normal platelet counts by human standards. Since

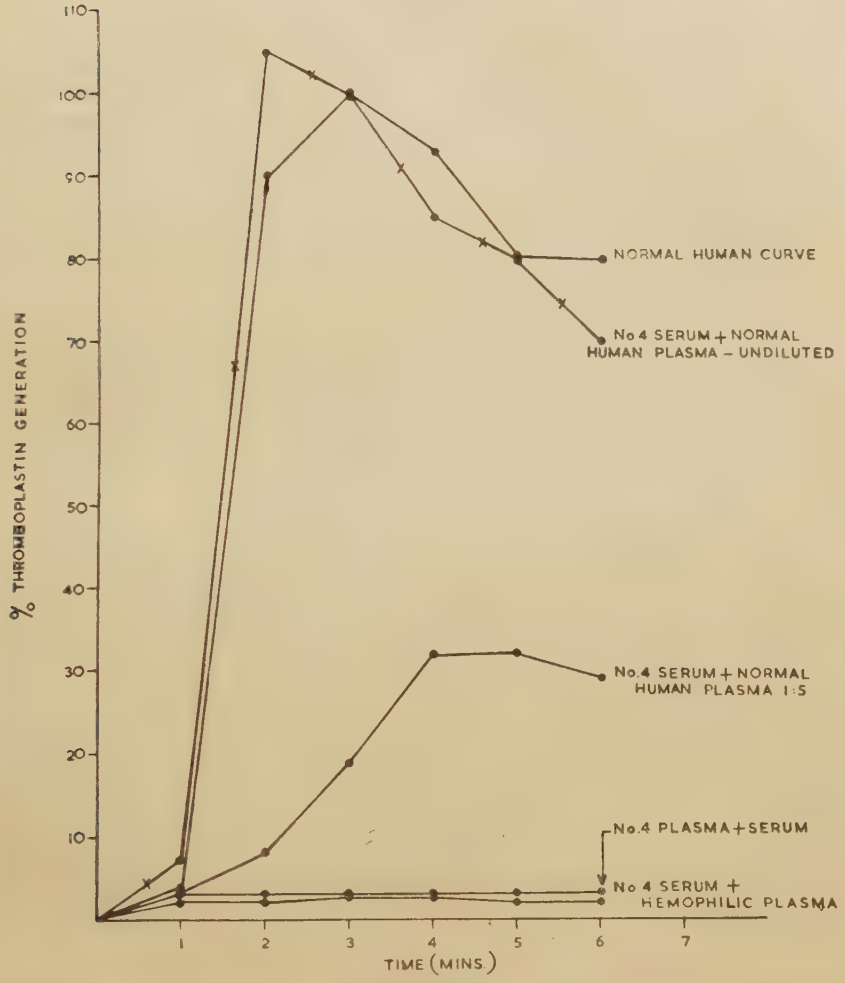
TABLE 1

RESULTS OF COAGULATION TESTS IN 12 HORSES

Breed	*Sex*	*Age (yrs.)*	*RBC (mill./cmm.)*	*WBC (thous./cmm)*	*Clotting time Glass (min.)*	*Silicone (min.)*	*Clot retraction(%)*	*Platelet (thous./cmm)*	*1-stage prothrombin (% of human normal)*	*Factor VII (% of human normal)*	*Factor V (% of human normal)*	*Heparin (gamma protamine)*	*Thromboplastin Generation (% of human normal)*
1. Thoroughbred	♀	14	7-92	9	26	57	3	175	43	74		80	
2. Thoroughbred	♂	3	5.00	12.8	32	86	4	226	14	47		80	
3. Thoroughbred	♂	9	6.78	4.5	27	84	13	209	14	59		100	
4. Shetland Cross Pony	♀	1	8.32	14.4	24		12	223	30	76		80	3
5. Shetland Cross Pony	♂	1	8.81	16.1	23		8	276	30	63		80	
6. Shetland Pony	♂	2	7.63	7.2	20		7	238	28	69		120	12
7. Thoroughbred	♀	18	6.87	8.8	26		5	160	35	63		120	
8. Hunter	♀	23	7.40	8.9	26		6	—	32	71		120	3
9. Thoroughbred	♀	16	—	—	22		11	—	46	91		140	
10. Thoroughbred	♀	5	10.75	6.1	25		15	132	46	93	116	140	12
11. Hunter	♂	5	6.60	10.8	26		16	173	33	100	135	140	12
12. Thoroughbred	♀	5	8.55	13.6	22		11	151	37	100	150	140	11

FIG. 2

THROMBOPLASTIN GENERATION IN THE HORSE AND ITS CORRECTION BY NORMAL HUMAN PLASMA



clot retraction has been shown to vary directly with the "adhesive" platelet count (Still, 1952), this test was performed by the method of Mootlen and Vroman (1949) on the first two horses studied in Table 1. The adhesive platelet count was 90,000 and 50,000 respectively, i.e. within the normal human range. Therefore, it appears that abnormal platelet function cannot fully account for the defective clot retraction. Unfortunately, studies of fibrinogen levels were not possible. These would also have been of value in investigation of the prolonged one-stage prothrombin times, since neither Factor V or

VII reduction appears to be the responsible factor. Two stage prothrombin determinations were not carried out.

It would appear that a deficiency of anti-haemophilic globulin is the normal state in the horse and is present in both sexes. The only other animal reported to show similar changes is the dog. Brinkhouse and Graham (1950) have bred a group of "haemophilic" dogs which exhibits spontaneous bleeding in the form of hemarthroses and mediastinal haemorrhage. However, this is a carefully in-bred group of abnormal dogs. From the level of the clotting time and thromboplastin generation, the horses studied would correspond to moderately severe human haemophiliacs. Because of the rarity of spontaneous haemorrhage in the horse, there must be compensatory mechanisms in the horse to account for the rarity of pathological haemorrhage. A possible explanation may be an increase in the tissue thromboplastic activity.

These findings may explain the success of Soulier and Larrieu (1953) in using horse plasma as a suitable platelet and thromboplastin-free substrate for tests. They tried plasma from several species of animal but found the horse most satisfactory. They state that the plasma retained 60 per cent of its anti-haemophilic activity when subjected to prolonged centrifugation in the cold but, apparently, they were not impressed by the pre-treatment level.

The significance of these findings will be of importance not only to those interested in investigational work on human haemophilia but also to those who are interested in species differences. Human or dog haemophilic plasma forms the necessary substrate for one of the principal methods of assay of anti-haemophilic globulin in use today. Since these are not generally available, the use of horse plasma either in the above mentioned method or in the thromboplastin generation test makes possible an efficient in vitro method of testing substances for anti-haemophilic activity. In this connection, Murray, Johnson and Seegers (1954) have recently reported that histamine and certain anti-histaminics appear to have anti-haemophilic activity in vitro. We were unable to confirm this finding using horse plasma and Benadryl (anti-histaminic) in the thromboplastin generation test.

CONCLUSIONS

Twelve horses, free from apparent haematological disorders, were studied by means of newer coagulation tests. In comparison to known human values, the most significant findings were found to be a prolonged clotting time, poor clot retraction despite a relatively normal platelet count and a prolonged one-stage prothrombin time.

By means of the thromboplastin generation test, it was found that the prolonged clotting time was due to a deficiency of anti-haemophilic globulin. The defect was present in both sexes and was

fully corrected by normal human plasma. This finding is of interest since no other known animal exhibits this defect and since it offers a useful tool for those interested in investigating haemophilia in man.

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CRYPTOSPORIDIUM MELEAGRIDIS (SP. NOV.)

By

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INTRODUCTION

In 1910, Tyzzer described a coccidium found in the stomach glands of tame mice which he named *Cryptosporidium muris*. The life cycle closely approximated that of the commoner known coccidia but there were important differences which justified the creation of a new genus. The parasite did not invade the host's tissues but lived its entire life cycle on the epithelium of the gastric glands and no sporocysts were formed, four sporozoites being found free in the oocyst. The oocyst did not require to undergo any further developmental stages outside the host's body but sporozoites could emerge from the oocyst within the stomach glands and start a new reproductive cycle.

In 1912, Tyzzer described another species, *Cryptosporidium parvum*, which he found on the villus epithelium throughout the small intestine of mice from the duodenum to ileum. He showed the two organisms were separate species by feeding experiments; the stomach parasite never affected the small intestine and vice versa. Both organisms were found throughout the year in mice of all ages and either could be present in enormous numbers in apparently healthy mice.

PRESENT INVESTIGATION

For over two years a close study has been made at Lasswade of Hexamitiasis in turkey poults (Wilson and Slavin, 1955). Bowel scrapings have always been used as the inoculum for further infections. In three separate series of transmission the parasite, *Hexamita meleagridis*, has progressively dropped in virulence towards the 20th passage and failed to cause serious illness until, at about the 23rd passage, the strain has been lost through failure to infect susceptible, fourteen day old poults.

In May of this year the third strain was lost. Weekly visits were made to the affected farm in the hope of picking up a fresh virulent strain. Loose droppings were collected from the brooders, mixed in broth and given by mouth to 14 day old poults in groups generally of ten. At this time the farmer was suffering moderate losses from diarrhoea in birds 10 to 14 days old. Animal parasites recovered from the droppings and from sick birds included a new species of *Histomonas* found in the caeca, *Trichomonas*, *Chilomastix*, *Eutrichomastix*, *Strongyloides* and the new parasite described in this article and provisionally named *Cryptosporidium meleagridis*.

The parasite was found in enormous numbers on the villus epithelim of the terminal third of the small intestine both in smears and in sections. In one heavily infected case sections were taken throughout the intestine and only this portion was found affected. As the accompanying illustrations show, all stages of the life cycle except the sporozoites have been found. All the other stages conform so clearly to Tyzzer's excellent account (1912) of the Cryptosporidium of mice that he might have been describing the parasite found in turkeys here

Cryptosporidium meleagridis is a coccidium parasitic on the epithelium of the small intestine of turkeys; it has so far been found only in the terminal third portion. It is a typical member of the sporozoa having an asexual cycle with production of schizonts and merozoites and a sexual cycle with production of microgametes and macrogametes, zygote and oocyst. Sporozoites have not been seen but it can be presumed, from the organism's otherwise close uniformity with Tyzzer's murine species, that four sporozoites are formed.

As Tyzzer found with *C. muris* and *C. parvum*, morphological study of *C. meleagridis* has been difficult as the organism is so small and stains only faintly by methods which reveal the finest detail in other protozoa. The description given here is based on the appearance of the parasite in dried smears stained by MacNeal which is probably the most intense of the Romanowsky modifications.

Merozoites. These are slender and falciform, 5.0μ long $\times$ 1.1μ at their greatest width. They taper towards the ends, the head end being blunter than the tail end. The nucleus is oval and subterminal and stains a faint pink, while the cytoplasm stains a faint blue. The nucleus does not appear to possess much chromatin and its central area is frequently unstained. No other chromatin structure has been seen in the merozoites. The probable stages from merozoite to trophozoite or gametocyte have been traced. The organism shortens and broadens but the nucleus remains situated towards one end. Tyzzer found that the merozoites attached themselves to the host cells by the tail end or narrow end furthest from the nucleus.

Trophozoites. These are roughly oval in shape, average between 4.0μ and 5.0μ at their greatest diameter and have an eccentric oval nucleus which stains a faint pink and is frequently difficult to discern. In the small trophozoites the cytoplasm stains light blue but in the larger ones it is slatey blue. In heavily infected sites the trophozoites are attached to the epithelium of the villi in enormous numbers and almost form a covering sheath (Figs. 18, 19, 20, 21). Tyzzer found that the trophozoites had a small projection on one side which penetrated the striated border of the epithelial cell and presumably had some absorptive function. This appearance has been observed in sections of infected bowel of turkeys,

(organism at extreme right of Fig. 18), but not in stained smears. In turkeys also, trophozoites have been seen in the goblet cells and between cells as far down as the basement membrane (Figs. 21, 14). In lightly infected sites the trophozoites are found in the surface depressions between epithelial cells (Fig. 20).

Schizonts. The exact method of chromatin division whereby a schizont containing eight merozoites is formed is not clear but by analogy with other coccidia can be presumed to be a series of three double divisions. The earliest identifiable schizont is an oval body 4.0μ × 5.0μ with eight evenly dispersed small chromatin bodies. Other forms can be seen which are almost certainly of the schizont series with two and four chromatin divisions. The chromatin is lightly stained in comparison to that of the microgametocytes. Eight merozoites are formed.

Sexual Cycle

Macrogametocytes. These are roughly oval structures 4.5μ to 5.0μ × 3.5μ to 4.0μ. They have a slatey blue cytoplasm with a tendency to very fine vacuolation and a faintly staining eccentric nucleus. It has sometimes been impossible to discern the nucleus. Macrogametocytes have been identified in the present work by presuming that all organisms with a single superimposed microgamete must be the female element of the sexual cycle (Fig. 16).

Microgametocytes. These are rounded or oval structures generally about 4.0μ in their greatest diameter with sixteen intensely stained reddish purple rods around the periphery, most of which are radially arranged. The exact system of chromatin divisions which produces this is not clear but appears to be a series of four double divisions. Microgametocytes have been seen on the surface of the epithelial cells (Fig. 20), deep in the mucosa (Fig. 14), within the nucleus of a cell (Fig. 13) and seeming to emerge from the nucleus. With regard to Figs. 12 and 13 it is interesting to note that *Dobellia binucleata* (Ikeda, 1914) and *Cyclospora karyolytica* (Schaudinn, 1902) both invade cell nuclei as essential stages in the life cycle.

Microgametes (Figs. 10 and 11). These are small solid chromatin rods 1.0μ × 0.3μ with rounded ends and stain intensely reddish purple. Of all the stages of the parasite found, these are the only ones which when stained give a satisfactory clear picture comparable to that given by other protozoa. No flagella have been observed by methods which clearly show the flagella of *Eimeria* microgametes and probably none exist. This is discussed later.

Oocysts (Fig. 18). These are oval, 4.5μ × 4.0μ with a very foamy cytoplasm and a faint, poorly defined wisp of nucleus placed eccentrically. In smears they are surrounded by a thin unstained area which can be regarded as the cyst wall. Tyzzer showed that the vacuoles in the oocysts were food vacuoles by staining them with iodine. Oocysts with sporozoites have not been found in the present

work and Tyzzer's explanation of their absence in the case of *C. parva* infections is that as the zygote develops to the oocyst stage it quickly loses its hold on the epithelial surface and is swept along with the intestinal contents; hence not many sporulated forms would be seen in tissue preparations. Sporulation of the oocyst within the host is not prominent in *C. meleagridis* and although enormous numbers of oocysts have been seen in rectal smears no sporulated forms have been found.

In *Eimeria*, microgametes are produced in very great numbers by comparison with the macrogametes. They are also very actively motile by two long flagella arising from the broader end and when observed on a warm stage have a tugging, whip-like movement. Their flagella are easily demonstrable in stained smears, but even where they are not shown, as in thicker portions of the smears, the contortions and variety of shapes of the microgametes indicate that they are motile forms. The very active motility of the microgametes ensures that the macrogametes can be fertilised at some distance from where the microgametes arose. This is not true of all coccidia and some such as *Dobellia* and *Adelea* require both gametocytes to develop in very close proximity for fertilisation to occur and then contact is probably passively achieved by movement of the host's tissues. This juxtaposition of male and female elements is called syzygy and is characteristic of the *Cryptosporidium* described here. In smears it is very common to find developing microgametocytes and macrogametocytes close together in twos or in fours and Figs. 6 and 8 are typical pictures. It is common to find a microgametocyte beside a fertilised macrogamete. The latter have not been seen with more than one microgamete attached. The microgametes are most likely non-motile and their unvarying shape confirms this. There are no known protozoa, whether flagellated or not, which do not alter the shape of the body during movement.

From Tyzzer's work with the murine cryptosporidia it seems unlikely that this *Cryptosporidium* is a cause of severe economic loss and it was not so in the present cases. There is no doubt that the organism can kill but the usual picture of heavy infections is diarrhoea and unthriftiness in the first weeks of life. The disease can be diagnosed only with difficulty in dead material sent to the laboratory; a similar difficulty was experienced by Tyzzer in distinguishing the organism from cell debris, in mice which were not freshly dead. The most reliable method is probably the examination of stained smears of the rectal contents for oocysts (Fig. 17).

CONCLUSIONS

Illness with diarrhoea and a low death rate in 10 to 14 day old turkey poults is shown to be caused by a coccidium which does not invade the host's tissues but completes its life cycle on the epithelium of the villi of the small intestine. The various stages of the life

history are described. The coccidium has been tentatively designated "*Cryptosporidium meleagridis*".

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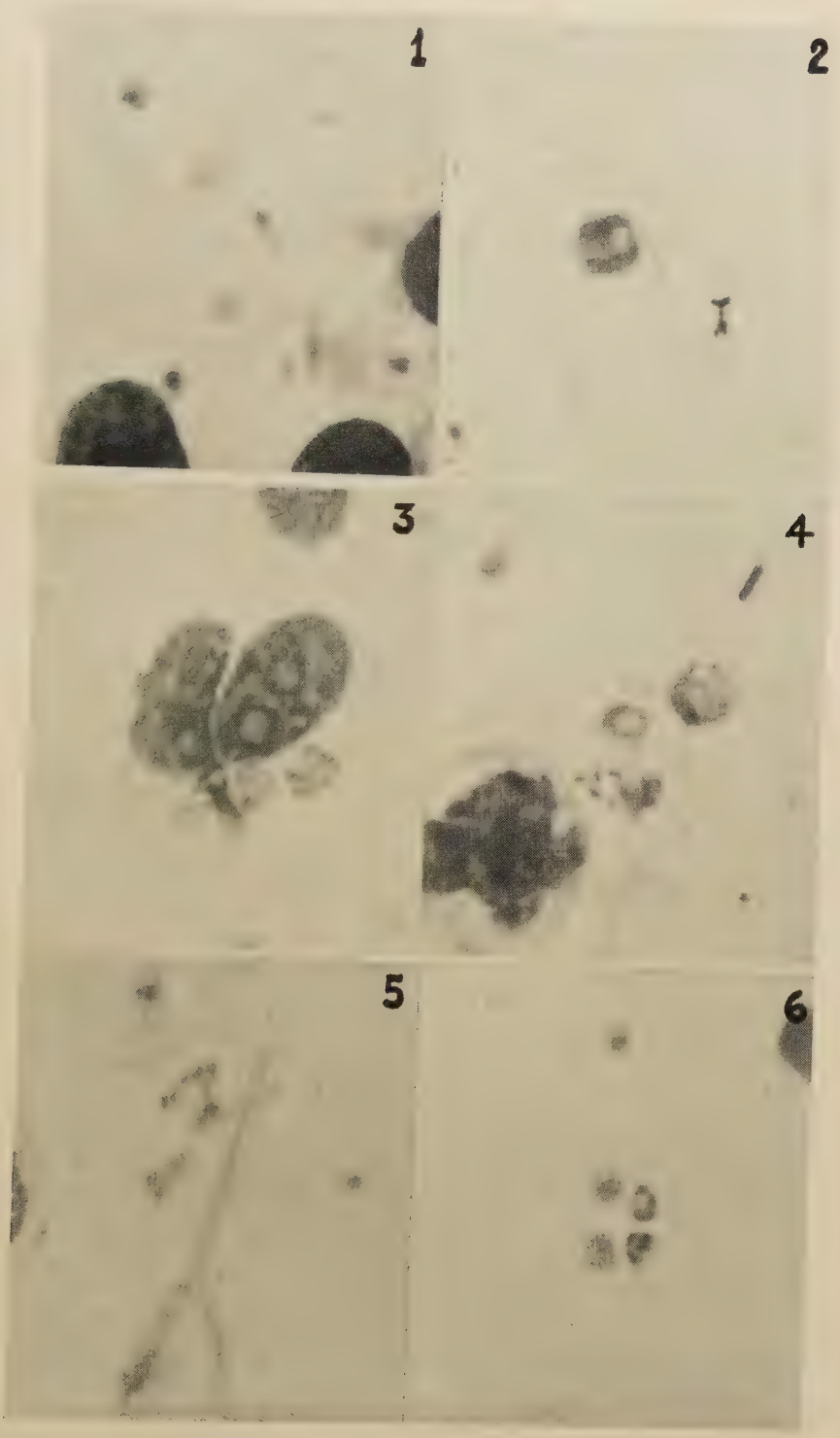
LEGEND TO TEXT FIGURES

Fig. 1. A single merozoite: × Ca 2000.
„ 2. A single schizont of four divisions: × Ca 1500
„ 3. A schizont of eight divisions in the asexual cycle with fertilised macrogametocyte beside. The rod on the latter is the microgamete: × Ca 1500.
„ 4. Microgametes with fertilised macrogametocyte and trophozoite: × Ca 2500.
„ 5. Schizont with eight almost mature merozoites.
„ 6. Microgametocyte (right top) with four divisions, schizont (bottom right) with four divisions and two trophozoites: × Ca 1300.
„ 7. Microgametocyte with eight divisions. The early peripheral position of the chromatin division is seen: × Ca 1500.
„ 8. Microgametes (top left), schizont of eight divisions (bottom), trophozoite (left centre) and macrogametocye (right centre): × Ca 1500.
„ 9. Microgametocyte (top) and schizont with eight divisions: × Ca 1500.
„ 10. Microgametocyte with sixteen gametes ready to leave the cell: × Ca 1500.
„ 11. Microgametocyte with mature gametes: × Ca 1500.
„ 12. Schizont of four divisions in the free nucleus of an epithelial cell.
„ 13. Microgametocyte on edge of free epithelial nucleus: × Ca 1800.
„ 14. Microgametocyte deep in villus: × Ca 1500.
„ 15. Microgametocyte of eight divisions: × Ca 1500.
„ 16. Fertilised macrogametocyte with microgamete showing as a small rod: × Ca 1500
„ 17. Oocyst. The nucleus is shown as faint stained irregular structure at the bottom: × Ca 1500.
„ 18. Section of epithelium of villus showing Cryptosporidia along the free surface: × Ca 1200.
„ 19. Same subject as No. 18.
„ 20. Tangential cut of top of villus showing Cryptosporidia on the surface. A microgametocyte is seen at the right: × Ca 1200.
„ 21. Section of villus showing two Cryptosporidia within goblet cell: × Ca 1500.
„ 22. Cryptosporidia on surface of epithelial cells. Smear: × Ca 1500.
„ 23. Cryptosporidia on surface of epithelial cell: × Ca 1800.



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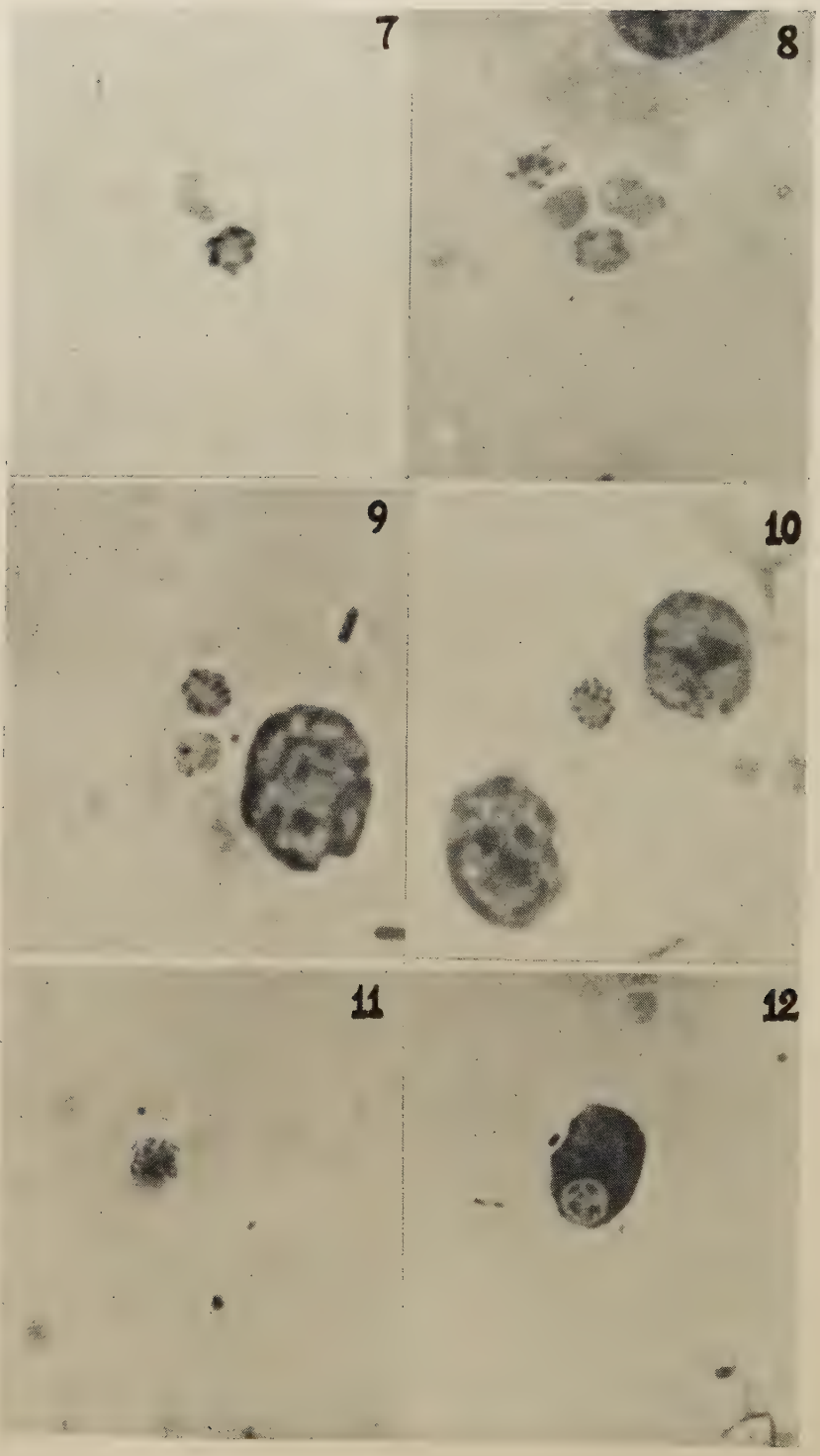
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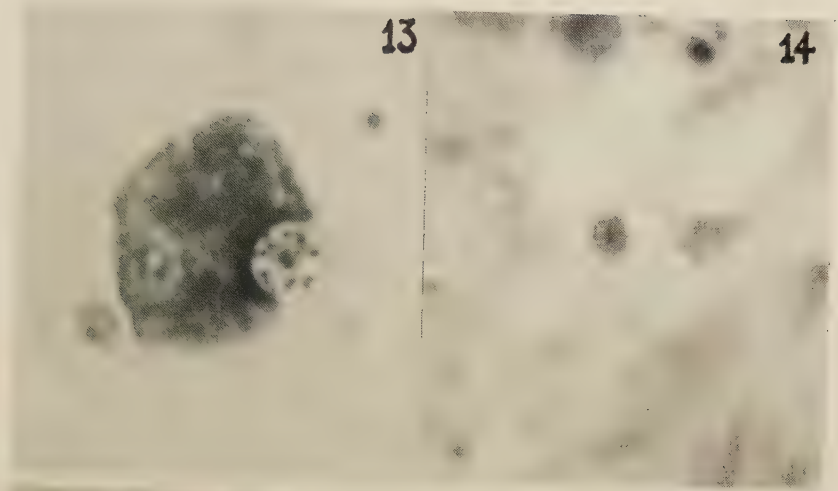
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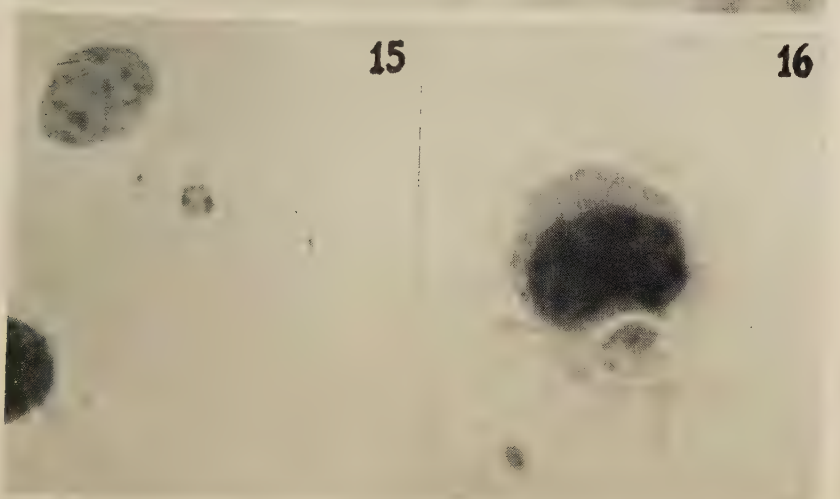
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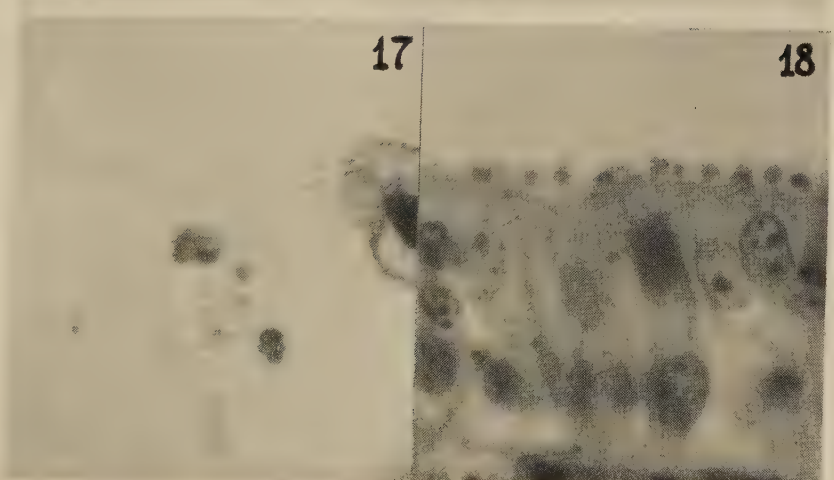
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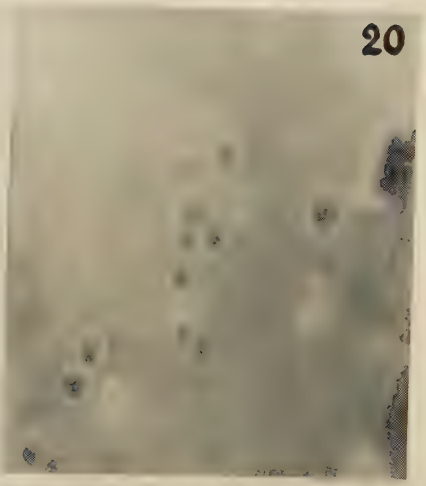
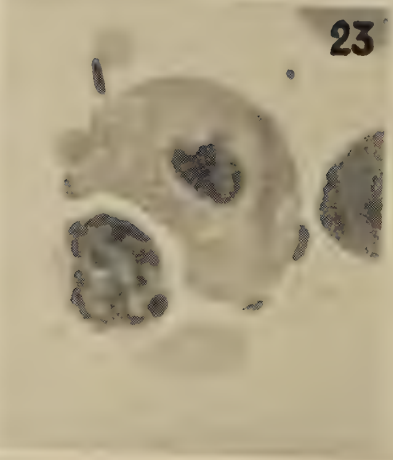
Cryptosporidium meleagridis





THE LONGEVITY OF *SALMONELLA GALLINARUM* IN THE FAECES OF INFECTED CHICKENS

By

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INTRODUCTION

In considering the control of an infectious disease such as fowl typhoid, it is important to have a reasonably accurate knowledge of the viability of the causal agent outside the body of the host.

Cultures of *Salmonella gallinarum* remain alive for considerable periods of time when exposed to various environmental factors (Biester and Schwarte, 1948; Orr and Moore, 1953). For example, Orr and Moore recovered this organism 93 days after it had been added to sterile tap water kept at room temperature, and 228 days after it had been placed upon sterilized sacking material. However, as far as the spread of the disease is concerned, the longevity of *Salm. gallinarum* in morbid material is of greater concern. Carcases of dead chickens are considered by several authors to be of importance in this respect, (Hall, 1946; Jordan, 1954; Sellers and Harbourne, 1954). Jordan isolated *Salm. gallinarum* from the liver of chickens up to 11 days after they had died from typhoid and from the bone marrow up to 32 days. Orr and Moore had also isolated this organism from liver that had been kept at 7°C. for 14 days after removal from an infected chicken. Experimental evidence of the part played by infected faeces in the spread of typhoid has been provided by Gordeuk, Glantz, Gallenbach and Thorp (1949). However, little or no detailed observations have been carried out on the survival time of *Salm. gallinarum* in infected faeces despite the fact that whatever vehicle of infection is under consideration the immediate environment of *Salm. gallinarum* in such material will in most instances inevitably be faeces and the conditions pertaining in the latter will have an important effect on the survival time of the *Salm. gallinarum*. Since suitable material was readily available from infected chickens used in other experiments (Williams Smith, 1954 ; 1955a, b)it was decided to determine the survival time of *Salm. gallinarum* in faeces.

MATERIALS AND METHODS

Faeces. Faeces from chickens in all stages of fowl typhoid were employed. The birds had been infected 6 to 21 days previously by oral administration of 50 x 10^6 *Salm. gallinarum* bacteria (strain 9) in alkaline medium. The faecal material excreted by each chicken during a 6 to 12 hour period was mixed separately and then divided into 28 portions, each about the size of a normal dropping and weighing approximately 4 g. Half of these portions were placed, separate from each other, on a

wooden board in the inner compartment of a fold unit; the other half were similarly positioned but in the outer compartment, the whole being placed on a piece of open ground. Faeces of 8 to 10 chickens were studied at the same time. The whole series of experiments involving material from 76 chickens was carried out from September 1953 to June 1954.

Bacteriological examination. Portions of each faecal specimen from the inner and outer compartments of the unit were examined every 3 days. A large loopful of each was cultured directly on to a plate of desoxycholate-citrate-agar (D.C.S.S.) containing 1 per cent each of sucrose and salicin (Williams Smith and Buxton, 1951). The remainder of the portion was first incubated in 10 ml. of selenite-F medium (Hobbs and Allison, 1945) at 37°C. for 24 hours, and then sub-cultured on to D.C.S.S. medium. All plates of this medium were incubated at 37°C. for 24 hours and then examined for *Salm. gallinarum.*

RESULTS

Although the faecal specimens were collected from chickens in all stages of the disease, the degree of infection of the faeces with *Salm. gallinarum* was never heavy. At the preliminary examination, *Salm. gallinarum* was only found in 10 of the 76 specimens by direct culture and not at all at the first examination at 3 days or at any subsequent examination by this technique. By selenite enrichment, however, it was found to live for periods ranging from less than 3 to 37 days in the faecal specimens kept in the inner compartment of the fold units, the average survival time being 10.9 days. For those specimens kept in the outer compartment the range was from less than 3 to 31 days, the average being 8.9 days.

No correlation was noted between the time of the year when the examinations were carried out and the survival time of *Salm. gallinarum* although the general impression was gained that a prolonged dry atmosphere favoured survival.

The Effect of Moisture Content and Temperature on the Survival Time of Salm. gallinarum *in Faeces*

In view of the impression referred to above, faeces from an infected hen were divided into a number of equal portions which were then maintained at different temperatures indoors. Two portions were kept at each temperature; one was allowed to dry naturally and the other was kept moist by the daily application of sterile distilled water. The results of bacteriological examinations for *Salm. gallinarum* carried out daily, and of estimates of the total bacterial content obtained by plating out the specimens on agar 7 days after the commencement of the experiment (Table 1) show that *Salm. gallinarum* survived for much shorter periods in the moistened samples than in the others, the survival times being shorter at the higher than the lower temperatures; temperature had a much less noticeable effect on the unmoistened specimens. The shorter survival time of *Salm. gallinarum* in the moistened specimens was correlated

with a much higher total viable bacterial count of these than of the other specimens.

TABLE 1

THE EFFECT OF TEMPERATURE AND MOISTURE CONTENT ON THE SURVIVAL OF *SALM. GALLINARUM* AND OTHER BACTERIA IN INFECTED CHICKEN FAECES

Treatment of faeces	*Survival time (days) of Salm. gallinarum* at* 37°C.	30°C.	20°C.	15°C.
None	1	34	34	26
Moistened daily	1	1	6	13
	Total bacterial content			
None	+	+	++	++
Moistened daily	++++	++++	++++	++++

**Salm. gallinarum* was only found in these specimens by means of selenite enrichment.

A further 7 specimens, all from different infected chickens, were treated in the same manner as the faeces referred to above except that they were all kept at room temperature. The survival time of *Salm. gallinarum* in the untreated specimens were <3, <3, 8, 15, 15, 25 and 32 days. The survival times for the corresponding moistened specimens were only < 3, < 3, <3, <3, <3, <3 and 5 days respectively.

The Survival Time of Cultures of Salm. gallinarum *in Faeces*

The survival times of different strains of *Salm. gallinarum* in faeces of a healthy chicken are shown in Table 2. Broth cultures had been added at the commencement of the experiment in sufficient amount to give a concentration of approximately 10^7 *Salm. gallinarum* per

TABLE 2

THE SURVIVAL TIME OF DIFFERENT STRAINS OF *SALM. GALLINARUM* IN THE FAECES OF A HEALTHY CHICKEN

Treatment of faeces	*Survival time (days) of strain No.* 1	2	3	4	9
None	40	6	23	31	59
Moistened daily	7	6	6	2	2

Each specimen had approx. 10^7 *Salm. gallinarum* bacteria per gram added to it at the commencement of the experiment. They were kept at room temperature.

gram of faeces. The specimens were held at room temperature. *Salm. gallinarum* was recovered from them for periods of from 6 to 59 days, strain 9, the strain used for infecting the chickens in the previous experiments, surviving longest. *Salm. gallinarum* was not recovered from moistened specimens for longer than 7 days.

DISCUSSION

The results indicate that control measures in fowl typhoid must be based on the fact that *Salm. gallinarum* may survive in faeces for at least 37 days. The observation that *Salm. gallinarum* lived for much shorter periods in moistened faeces than in faeces allowed to dry and remain so may well have a practical application. The probable reason for this difference in survival time was that the conditions prevailing in the moistened specimens permitted considerable multiplication of bacteria other than salmonellae, as evidenced by the higher viable bacterial counts; this multiplication probably resulted in the production of substances inimical to the survival of *Salm. gallinarum*.

CONCLUSIONS

The average survival time of *Salm. gallinarum* in 76 faecal specimens collected from infected chickens was 10.9 days (range <3-37 days) when the faeces were kept in the inner compartment of a fold unit. The average for duplicate specimens kept in the open air was 8.9 days (range $<3 - 31$ days). Long periods of dry weather appeared to favour survival time, an observation that was confirmed experimentally.

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BILE PIGMENT METABOLISM IN CATTLE

Bile Pigments in Ox Bile, Intestinal Contents, and Faeces

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INTRODUCTION

Clinical jaundice is not of common occurrence in cattle. It has been observed as a feature of poisoning with *Senecio jacoboea* (ragwort) (Nicholson, 1945) and is one of the symptoms of delayed dimidium bromide poisoning (Plowright, Burdin and Thorold, 1952), in both of which conditions the primary lesion is in the liver, but severe hepatic lesions can be present without concomitant icterus (Garner, 1953). Again haemolytic disease in cattle is not constantly accompanied by jaundice; thus although pronounced in *Babesia bigemina* infection, it is not evident with *B. bovis* (Wenyon, 1926).

If excretion of bile pigments by the liver were affected, for example, by complete or partial obstruction of the bile ducts, then those bile pigments which are normally present in the bile would be expected to appear in the blood. In ox bile, according to many authors (*vide infra*), the principal bile pigment is not bilirubin but biliverdin. In cases of biliary obstruction in cattle biliverdin, rather than bilirubin, might therefore accumulate in the blood. In no known instance has the blood biliverdin concentration been studied in such conditions, observations being confined to the use of the van den Bergh reaction which, contrary to common belief does not determine biliverdin (Larsen, Evans and Watson, 1947). No biliverdin could be detected in the serum of normal cattle (Garner, 1953).

But is, in fact, biliverdin the chief pigment of ox bile? A search of the literature revealed a surprising situation. The pigments of cattle bile could not be decided upon, and even the colour noted varied from author to author. Colin (1873) gives the colour of bile as olive-green in the ox; Garrod (1900) quotes the Italian workers Zoja, Cavalli and Chiodora as stating that "the green bile of vegetable feeders" yields urobilin less readily than that of the carnivora owing to its relatively higher biliverdin content; Ellenberger and Scheunert (1925) record that the bile of ruminants is brownish green to dark green in colour and add that animals with green bile have oxidized the bilirubin to biliverdin; Dukes (1943) observes that "biliverdin is found in the bile of many animals, especially herbivores", and Marshall and Halnan (1945) ascribe the "yellow-green or dark green" colour of herbivorous bile to biliverdin. Ramsay (1946) states that fresh ox bile "is invariably a pure green colour" and records that "several attempts to prepare bilirubin from it (by precipitation as barium bilirubinate) were unsuccessful". He concluded that bilirubin plays no part in the normal metabolism of cattle. On the other hand, according to Lemberg and Legge (1949), fresh ox bile is usually yellow in colour and contains only bilirubin, while Martin (1949), in a study of the interactions of bilirubin with serum proteins, used bilirubin isolated

from ox bile. Further, Garner (1951) had no difficulty in preparing pure bilirubin from cattle bile by precipitation with barium chloride.

It was evident that bile pigment metabolism in cattle required further investigation, particularly as regards the relative importance of bilirubin and biliverdin in the normal catabolism of haemoglobin As a first approach to the problem fresh bile, intestinal contents, and faeces from healthy cattle have been examined for the presence of bilirubin, biliverdin, and possible derivatives of these compounds.

METHODS AND RESULTS

Bile

Bile was collected direct from the gall-bladders of slaughtered cattle and conveyed to the laboratory in tightly stoppered bottles filled to the brim. Absorption spectra were plotted using either the Beckmann or the Unicam spectrophotometer.

Bilirubin. All specimens examined gave a strong direct van den Bergh reaction.

Bilirubin was isolated (a) by extraction with light petroleum (b.p. 40-60°) from bile acidified with HCl (conc., 5 per cent v/v), (b) by adsorption on barium hydroxide (Hawk, Oser and Summerson, 1947), in each case followed by chromatography on alumina (Gray and Whidborne, 1946). The best preparation had a molecular extinction coefficient of 53,800 at 451mμ. This is in good agreement with the figures of 54,900 and 55,300 given by Heilmeyer (1931) and Martin (1949) respectively.

In chloroform solution, crude bilirubin from ox bile is destroyed very rapidly. This is illustrated graphically in Fig. 1, which shows the absorption spectrum of a fresh preparation and of the same solution after standing for 1 hour in a tightly stoppered 10 ml. volumetric flask. The difference curve corresponds almost exactly with the absorption of pure bilirubin in chloroform. This destruction may have been accelerated by light and by the presence of chlorophyll derivatives.

Biliverdin. Biliverdin could be obtained from bile diluted with ethanol (1:1 v/v) by either of two procedures (a) extraction with glacial acetic acid in ether followed by removal of the porphyrins with 0.54 per cent HCl, (b) extraction with ethyl acetate after removal of carotenoids and acidification with conc. HCl (5 per cent v/v). In order to minimize oxidative changes the extractions were carried out under nitrogen. The methyl ester of the biliverdin hydrochloride so obtained had m.p. 205-210°.

"*Urobilin.*" "Urobilin" is used here as a group term including stercobilinogen, stercobilin, mesobilirubinogen and mesobilene.

A chloroform extract of bile acidified with HCl was golden in colour and exhibited a well-marked absorption band at 498mμ. On addition of saturated ethanolic zinc acetate solution, a strong green

FIG. 1

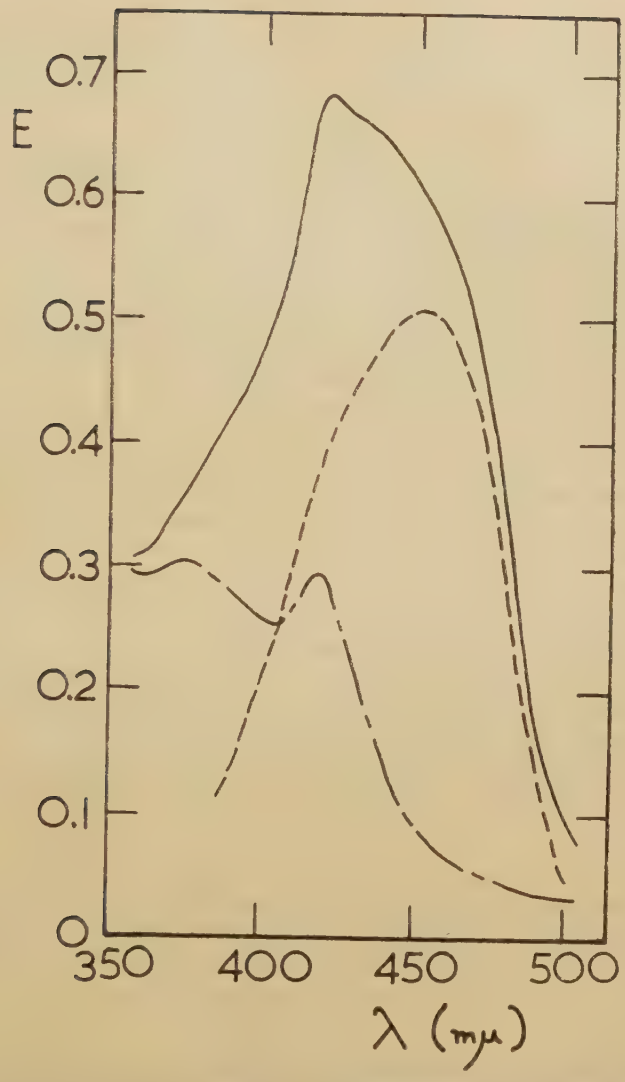

Fig. 1. Absorption spectrum of crude bilirubin preparation from ox bile in chloroform; ——, solution examined at once; — — — —, after 1 hr.; – – – –, subtraction curve corresponding to bilirubin destroyed during standing.

fluorescence was obtained and the absorption maximum shifted to 506mμ. That the pigment responsible for these phenomena was stercobilin and not mesobilene could be demonstrated by the fact that it was not oxidized by $FeCl_3$ (Lemberg, 1934). Attempts to obtain a pure preparation of stercobilin hydrochloride by the method of Watson (1934) were unsuccessful, the best preparation having a molecular extinction coefficient at 498mμ of 21,000 compared with 66,000 obtained by Heilmeyer and Krebs (1934).

Other pigments. During the course of extraction of biliverdin by the second procedure outlined above, a further bile pigment believed to be a biliviolin was isolated. The significance of this compound has been discussed by Garner (1954).

Various porphyrins were obtained from the ethereal acetic acid extracts, including phylloerythrin and other chlorophyll derivatives. Repeated fractionation of an 8 per cent HCl extract of the ethereal solution of porphyrins obtained from 20 litres of bile revealed the unequivocal presence of protoporphyrin (absorption bands at 502, 536, 576 and 630 mμ as compared with 502, 537, 576 and 632.5 mμ given by Bois, 1927) in addition to coproporphyrin. It is believed that this is the first time protoporphyrin has been described in ox bile. Paper chromatography indicates the presence of a third fraction, the identity of which is not yet known.

Intestinal Contents

The contents of the small gut were collected immediately after slaughter, filtered through muslin, then mixed with an equal volume of ethanol and again filtered.

Bilirubin. The aqueous ethanol solution was extracted with light petroleum until the extracts were colourless. The remaining solution was then acidified to approx. 5 per cent with conc. HCl and again extracted with petrol. Although there was no spectroscopic evidence for the presence of bilirubin in this extract, a chloroform solution of the brown solid obtained on evaporation of the petrol gave a fairly strong positive van den Bergh reaction. No evidence could be obtained for the presence of biliverdin or other bile pigments.

Faeces.

Neither bilirubin nor biliverdin could be identified in fresh faeces. Stercobilin hydrochloride was separated by the procedure of Watson (1934) and identified spectroscopically. A preparation with a molecular extinction coefficient at 498mμ of 35,000 was obtained.

DISCUSSION

There can be little doubt that bilirubin is, under normal conditions, excreted into the intestine of the bovine in the bile. The difficulty encountered by earlier workers in demonstrating the presence of bilirubin in cattle bile is probably explained by the ease with which the pigment is converted into various oxidation products, some of which are, spectroscopically, very similar to biliverdin. As stated in the text, in chloroform solution crude bilirubin from ox bile is destroyed. The bilirubin of human bile is readily susceptible to oxidation (With, 1945), and, as Lemberg and Wyndham (1936) remark, "when biliverdin is found in bile, its presence is sometimes due to post-mortem oxidation in the bile exposed to atmospheric oxygen." Haycraft and Scofield (1890) observed that

when ox bile was kept in tall cylinders its colour changed from green to brown and they claimed that the green pigment "biliverdin" (which they did not characterize) was reduced, even in the absence of putrefactive micro-organisms, to bilirubin. As this process took some months, it is unlikely that biliverdin was the origin of the bilirubin found in the present instance.

Apart from the demonstration of bilirubin itself in bile and intestinal contents, a product of its metabolism, in the form of stercobilin hydrochloride, has been found in both bile and faeces. As biliverdin, either under the action of intestinal bacteria *in vivo* (Garrod, 1900; Lemberg and Legge, 1949) or by reduction and subsequent oxidation *in vitro* (Watson and Schwartz, 1942) cannot give rise to stercobilin, it must be concluded that the latter is indeed formed from bilirubin present in the bile. The presence of quite large quantities of stercobilin in the bile suggests that there is an efficient enterohepatic circulation in the bovine. There is, in fact, ample evidence for such a circulation in the ruminant from the presence of phylloerythrin in the bile, since this porphyrin arises by degradation of chlorophyll in the gut (Quin, Rimington and Roets, 1935).

Bilirubin could not be identified unequivocally in the faeces (although petrol extracts gave somewhat doubtfully positive van den Bergh reactions), possibly indicating that the micro-organisms of the bovine gut are as active in reducing bilirubin to stercobilinogen as those in omnivorous and carnivorous animals.

The constant occurrence of biliverdin in ox bile is a rather more controversial matter. All methods used to extract the pigment involved acidification with hydrochloric acid, a process which was followed by either intensification of the green colour, if the bile were green already, as was the case with all specimens collected during the winter and early spring, or by a change to a pure emerald green in summer and autumn samples which were originally yellow-brown. This led to the suspicion that the green colour indicated rapid oxidation of bilirubin, but the amount of biliverdin found to be present in ethyl acetate extracts of winter bile seemed to be greatly in excess of what would have been expected from partial oxidation of the amount of bilirubin found in summer bile. Biliverdin is, therefore, probably normally present in winter bile and the almost immediate intensification of colour seen in such samples on addition of hydrochloric acid may be due to conversion of the pigment into its hydrochloride which is more absorbent in the red region of the spectrum. In summer bile, on the other hand, the weight of evidence is in favour of the greater part, at least, of the biliverdin arising by rapid oxidation of bilirubin. In these specimens development of the green colour was relatively slow and was probably analogous to the change in colour from yellow to green accompanying destruction of bilirubin observed in chloroform

solutions of crude bilirubin. The colour change was shown not to involve liberation of a coloured chlorophyll product from a leuco precursor (Noack and Kiessling, 1929).

The conclusion must be drawn that bilirubin is a normal constituent of ox bile, that biliverdin may not always be present, and that the course of breakdown of bilirubin in the gut of cattle is in all probability directly analogous to that in man.

CONCLUSIONS

Fresh ox bile normally contains bilirubin, coproporphyrin, protoporphyrin and stercobilin; it may not always contain biliverdin.

The presence of bilirubin, but not of biliverdin, has been demonstrated in intestinal contents.

The only bile pigment to be identified unequivocally in ox faeces is stercobilin.

It is concluded that the fate of the bile pigments in cattle is essentially similar to that in man.

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LEPTOSPIRA CANICOLA IN CATTLE

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INTRODUCTION

Leptospira canicola has so far been reported in the literature only as a cause of disease in man and dogs. In the literature very little is mentioned about infection of cattle by the canicola serotype of leptospira. During a severe outbreak of *L. canicola* infection among men, dogs and hogs in Georgia (U.S.A.) Starr (1953) observed significant agglutination in the blood of 2 out of 21 cows, which showed no symptoms of disease. Wellington, Ferris and Stevenson, (1953), in Australia, found low agglutinin levels (1 : 160) for *L. canicola* in a cow with haematuria.

Leptospira grippotyphosa was recognised as a cause of leptospirosis in cattle in Israel during the years 1949 to 1951 (van der Hoeden, Halevi and Dafni, 1953) The present report deals with outbreaks of disease among cattle caused by this particular serotype of leptospira.

OBSERVATIONS

Outbreak 1

In June 1949 the fresh carcase of a three weeks old calf which had died at the communal settlement (kibbutz) Naän was sent to the Veterinary Institute. It had been ill for two days, the main symptoms being extreme weakness, lack of appetite, bumping pulsation of the heart, and intense jaundice. Four weeks previously a similar case had occurred, the calf dying after three days of acute illness. From the kidneys of the first mentioned calf a strain of leptospira was cultivated in Korthof's medium (van der Hoeden, 1952).

From simple agglutination-lysis tests it was deduced that the Naän-strain was closely related to, or identical with, *L. canicola*, strain Utrecht Hond IV (Utr. H IV). It gave slight co-agglutination (1 to 6 per cent) against *L. icterohaemorrhagiae* A (strain Kantorovicz), *L. icterohaemorrhagiae* AB (strain Wijnberg), and *L. ballum* (strain S 102). For final identification, absorption-tests were carried out with the five above mentioned strains. Only the sera against *L. canicola* Utr. H IV and against the Naän strain were totally deprived of antibody in cross absorption tests with these strains. These results justified the conclusion that the Naän strain was serologically identical with *L. canicola*.

Outbreak 2

In December, 1952, in Kibbutz Givat Brenner, 6 km. from Naän, a five months old calf showed lack of appetite, dullness, pale mucous membranes, reddish urine, and a temperature of 39.8°C. or more

for 4 days. Clinical recovery occurred in 14 days. Blood taken on the sixth day of illness gave negative results in agglutination tests with *L. icterohaemorrhagiae* and *L. grippotyphosa*. but an agglutination-lysis titre as high as 1 : 20.000 was obtained with *L. canicola* (strains Utr. H. IV and Naän).

The cattle in the Settlement were quartered in four different sheds. The adult cows were kept in shed 1 and their calves transferred to a separate shed 3 to 5 days after birth. After 2 to 4 weeks they were sent to shed 2, where they remained for 5 to 6 months. The heifers were then kept in shed 3 until near to calving. The sick calf came from shed 3.

Preliminary examination of the sera of 140 adult cattle in shed 1 and 38 calves in shed 3 (Table 1) showed a widespread infection in the latter. The significance of the positive findings was checked by testing the sera of 556 cattle from 16 different places in the country between December, 1952 and January, 1954. Only 3 showed a titre as high as 1 : 200. An agglutination level above 1 : 200 has therefore been considered significant.

TABLE 1

BLOOD EXAMINATION CATTLE OUTBREAK 2

Date	*Shed*	*Total number examined*	*Agglutinin titres with* L. canicola				
			negative	*1 : 20*	*1 : 200*	*1 : 1000*	*1:2000—1:20.000*
12.12.1952	1	140	134	5	0	1	0
12.12.1952	3	38	2*	3	5	19	9

*On 29.1.1953 the blood of one of these animals agglutinated *L. canicola* to a titre of 1 : 2000.

Since the agglutination of *L. canicola* might be due to cross reactions with antigenically related leptospira, additional tests were done. Only the canicola type strains (Utr. H IV and Naän) were agglutinated at high titres (1 : 2000 to 1 : 20.000). *L. icterohemorrhagiae* and *L. ballum* were slightly agglutinated (1 : 20 to 1 : 200), while *L. autumnalis. L. pomona, L. sejröe, L. saxkoebing, L. hyos* and *L. grippotyphosa* were not agglutinated. Furthermore absorption tests carried out with these cattle sera and suspensions of the strains canicola Utr. H IV, canicola Naän and ballum showed that the agglutinins present were identical with those of the canicola serotype and different from the ballum type (Table 2).

Clinical data and general condition of the calves. During November and December, 1952, approximately one third of the young calves became sick, and nearly half of these died or had to be slaughtered.

TABLE 2

SERUM ABSORPTION TESTS

Agglutination titre with :	*Unabsorbed*	*Serum of calf* 348		
		Absorbed by L. canicola *Utr. H IV*	*Absorbed by* L. canicola *Naän*	*Absorbed by* L. ballum *S102*
L. canicola Utr. H IV	3200	0	0	3200
L. canicola Naän	3200	0	0	3200
L. ballum S102	400	0	0	0

A few showed redwater, but neither Theileria, Anaplasma or Babesia could be detected. Several calves showed only lack of appetite, dullness, and rise of temperature during one or a few days. Recovery often occurred within a week and, as a rule, high titres against *L. canicola* were found in these cases. The general health of the young stock had deteriorated for a year or two, the average daily milk yield of the heifers being about half the normal, but from April, 1953 the condition of the calves in sheds 2 and 3 improved.

Spread of the infection. Shed 1. The only positive reactor in the 140 cattle in shed 1 (table 1) was a cow which had recently been in poor condition showing dullness, no appetite, and diminished milk yield. Her serum agglutinated *L. canicola* (Utr. H IV and Naän) to dilutions of 1 : 1000 on each of three bi-monthly tests. No agglutinins were found for several other leptospira serotypes. On April 22, 1953 she had a premature calf, and the placenta was retained. The agglutination titre for *L. canicola* was now 1 : 100.

On April 21, 1953 agglutination tests were repeated on 116 of the 139 negative cows, and 111 were again negative, the other 5 having tires between 1 : 20 and 1 : 200. Another 35 cattle in shed 1, which had not been examined previously, were also tested, 9 showing significant titres. All 9 were heifers recently transferred from shed 3 to shed 1.

New-born calves of reacting mothers showed leptospira-agglutinins in their blood but repeated tests revealed that their titres dropped gradually, e.g. in one case from 1:4000 to 1:20 in 3 months.

Shed 2. Twenty-five out of 72 calves in shed 2 showed significant titres on one or more dates, 22 of them having an agglutinin level of 1 : 2000 or higher. Most of the positive reactions were found among the older calves, their blood titres often rising with the length of time they were in the shed. Most infections were observed prior to February, 1953, and after that time they occurred only sporadically. None of the 33 calves, born in July, 1953 or later, showed agglutination although they were tested repeatedly until December, 1953.

Shed 3. Sera of 127 out of 186 calves and heifers in shed 3 agglutinated *L. canicola* to significant titres, 43 of them in dilutions

of 1 : 2000 or higher. In several cases the titre increased; in others it remained constant or dropped during their stay in shed 3.

Since no canicola agglutinins were found in heifers of shed 1 which had been transferred from shed 3 earlier than January, 1953, whereas several heifers which had entered shed 1 on a later date showed positive reactions, it seems that the canicola infection in shed 3 started not long before December, 1952. Some of the calves in shed 3 may have been infected before they were transferred from shed 2. With the exception of the heifers which were moved from shed 3 after December, 1952, less than one per cent of the animals in shed 1 reacted to *L. canicola*. On the other hand 58.8 per cent of the 258 calves and heifers of sheds 2 and 3 showed positive reactions between December, 1952 and August, 1953. After this date no new infections were observed, either clinically or serologically. Thus the enzootic which started about November, 1952 subsided in July, 1953.

Outbreak 3

In May, 1953 blood, milk and urine of a cow, from a private dairy farm in Yazur, which had been ill for several days, were sent to the Veterinary Institute. The blood serum was brownish-red, the urine almost black. The milk had become thick and yellow, and its amount had decreased to less than $\frac{1}{2}$ litre. The body temperature which registered 40°C. on 29.5.53, dropped to 38.8C°. two days later. Meanwhile the general condition improved, and finally the urine regained its normal appearance. Piroplasms could not be detected in blood smears. *L. canicola* was agglutinated by the blood-serum in a dilution of 1 : 4000, by the milk whey in 1 : 160, but not by the urine. No agglutinins against *L. icterohaemorrhagiae* and *L. grippotyphosa* were found in the blood of the sick cow, or in that of 11 apparently healthy cows in the same yard. One of the latter, however, had a serum titre against *L. canicola* of 1 : 2000, and another 1 : 400. The whey of both these reactors agglutinated the same strain to a dilution of 1 : 160.

One of two calves showed an agglutinin titre of 1 : 8000 for *L. canicola,* without perceptible symptoms of disease.

Outbreak 4

Blood of a sick cow from a private farm at Beit-Dagon near Yazur, was sent for examination in February, 1954. On the preceding day the milk yield had considerably decreased, the milk being yellow and cheesy. The body temperature rose to 41°C. The urine was red and the mucous membranes were yellow. No piroplasms were found in blood films. The blood serum agglutinated *L. canicola* in a dilution of 1 : 8000 and the whey in a dilution of 1 : 160. No agglutinins were present against other leptospira serotypes.

Almost simultaneously a second cow showed redwater and decreased milk yield during two days; its canicola serum-titre was 1 : 32.000. Seven of the 15 apparently healthy adult cows in the same shed showed high serum titres for *L. canicola* (1 : 1000 to 1 : 16.000) and significant levels were also found in the whey (1 : 160 to 1 : 320). Three calves did not show antibodies.

On one of two other adjoining farms a cow was ill with similar symptoms in March, 1954. Two days later serum tests revealed no agglutinins against *L. canicola* or *L. grippotyphosa*, but 18 days later a positive reaction for *L. canicola* in a dilution of 1 : 1000 was found. Blood examinations of all cows on this farm were then carried out when one reacted with *L. canicola* in a dilution of 1 : 200, two of 1 : 1000, three of 1 : 2000, two of 1 : 4000, one of 1 : 16.000: three were negative.

No disease occurred among the 5 cattle on the adjoining farm. Three had no agglutinins either in the blood or in the milk. Two others (a one year old calf and a heavily pregnant adult cow) showed canicola agglutination in dilutions of 1 : 1000 and 1 : 2000 respectively. On 5 farms in the vicinity of the 3 infected herds 3 out of 26 cows showed significant serum titres against *L. canicola* (1:2000, 1 : 4000, 1 : 16.000). On 9 remote farms in the same village none of 26 cows reacted.

Experimental Infection

A $3\frac{1}{2}$ months old calf was infected by dropping in the eyes 0.5 ml. of a *L. canicola* culture which had recently been isolated from a dog at Givat Brenner (third subculture, on seventh day of growth). From the seventh to the tenth day after instillation of the culture, the temperature rose to 41°C. Specific serum agglutinins reached a titre of 1 : 8000 after 18 days. On the twentieth day the calf was slaughtered. Macroscopic and microscopic examination revealed acute and subacute focal interstitial nephritis and the presence of clumps of leptospira in the lumina of the tubules. The urine in the bladder showed many active leptospirae.

Epidemiology

On 3 of the infected farms there was no evidence to suggest that dogs were the source of infection of the cattle. However, several jackals which were encountered in the near vicinity of the infected sheds were excreting *L. canicola* in their urine and it is most probable that this species was the main reservoir from which cattle contracted the infection (Van der Holden 1955).

None of the 27 workers in the infected cowsheds showed antibodies against *L. canicola* in their blood.

CONCLUSIONS

During 1949 to 1951 many cattle suffering from leptospirosis in Israel were infected by the *L. grippotyphosa* sero type (6). From 1952 onwards the picture changed in so far as all cases occurred in a different area and were caused by *L. canicola.*

In 4 herds the canicola infection caused fever of short duration, lack of appetite, jaundice, haemoglobinuria and decreased milk yield. In the majority of cases however the infection remained clinically inapparent. Abortion seemed to be associated with *L. canicola* infection in heifers.

L. canicola was cultivated from the kidneys of a calf which died after a short illness.

Calves born from reacting mothers showed high agglutinin titres against *L. canicola* which persisted for several months. In addition the milk of infected cows often contained agglutinins for *L. canicola* in lower titre.

A recently isolated canine strain of *L. canicola* dropped into the conjunctival sac of a calf induced an interstitial nephritis, leptospira being excreted in the urine.

The main source of infection of cattle was presumed to be the jackal.

No infections were observed among persons handling the infected cattle.

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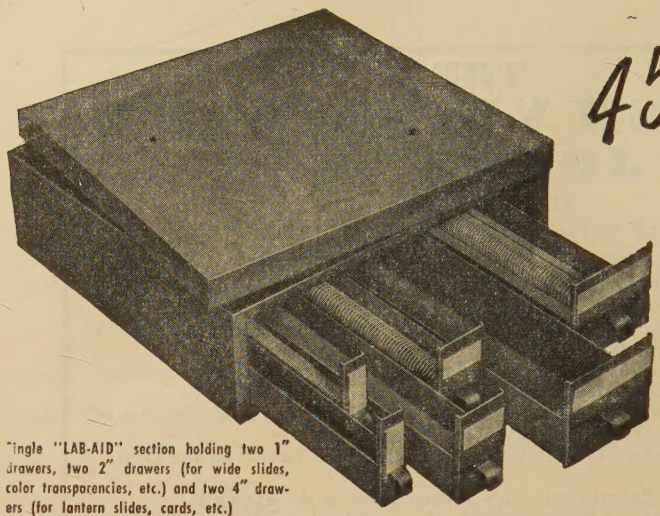


Single "LAB-AID" section holding two 1" drawers, two 2" drawers (for wide slides, color transparencies, etc.) and two 4" drawers (for lantern slides, cards, etc.)

45% greater capacity

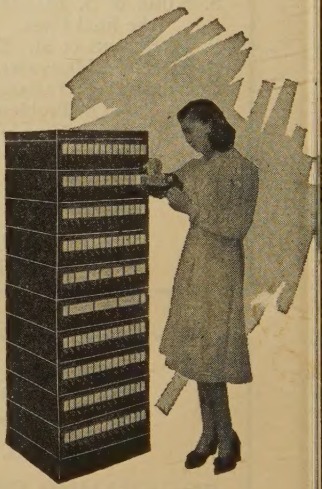
cuts filing costs . . .

saves floor space

How can a Technicon "LAB-AID" filing cabinet measuring only 19" square by 5" high hold up to 6500 (average) one-inch microslides? The single section illustrated here with its drawers extended will show you why . . . it's because there are no thick bulkheads between drawers — no space-consuming buttresses, no bulky tracking guides. Yet, close-set as they are, every drawer slides straight and true . . . easily, smoothly, without binding.

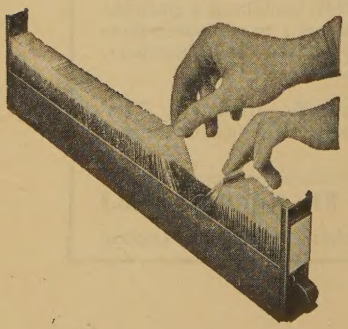
Drawers are freely interchangeable, too, thanks to the unique "LAB-AID" unit-gauge track system, so that you can intermix 1" drawers with 2" drawers (even 4" drawers, if you like) according to your needs.

Staunch, fireproof "LAB-AID" units are strongly built of heavy gauge steel for a lifetime of satisfactory use. Beautifully finished in olive green enamel, with combination drawer-pulls and label holders. Separate bases and finishing cap panels make complete units of any number of sections your filing needs require, expandable at will at any future time.



A stack of ten sections, complete with base and cap panel. Interlocking design lets you pile sections right up to the ceiling. Staunch "LAB-AID" construction easily bears the heavy weight of solidly packed slides.

For filing current slides, the removable spring-separator lets you riffle through them as easily as you flip the pages of a book . . . easy to file, easy to find. Slip it in when you need it, take it out when you use the drawer for storage filing.



TECHNICON

Lab-aid laboratory filing cabinets

Technicon "LAB-AID" filing cabinets have long been standard filing equipment in laboratories throughout the world. Now made in France, they can be easily imported into the United Kingdom.

COMPAGNIE TECHNICON
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